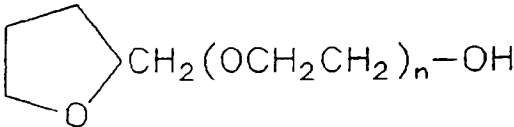




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 47/10, 9/06, 9/72		A1	(11) International Publication Number: WO 91/16929 (43) International Publication Date: 14 November 1991 (14.11.91)
(21) International Application Number: PCT/DK91/00119 (22) International Filing Date: 3 May 1991 (03.05.91) (30) Priority data: 1170/90 10 May 1990 (10.05.90) DK 2075/90 30 August 1990 (30.08.90) DK (71) Applicant: NOVO NORDISK A/S [DK/DK]; Novo Allé, DK-2880 Bagsvaerd (DK). (72) Inventors: BECHGAARD, Erik ; Valbirkvej 6, DK-2900 Hellerup (DK). GIZURARSON, Sveinbjörn ; Vesturgata 38, IS-230 Keflavik (IS). HJORTKJÆR, Rolf, Kühlman ; Ved Hegnet 19, DK-3050 Humlebæk (DK).		(74) Agent: NOVO NORDISK A/S; Patent Department, Novo Allé, DK-2880 Bagsvaerd (DK). (81) Designated States: AT (European patent), AU, BE (European patent), BG, BR, CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LU (European patent), NL (European patent), NO, PL, RO, SE (European patent), SU. Published With international search report.	
(54) Title: A PHARMACEUTICAL PREPARATION CONTAINING N-GLYCOFUROLS AND N-ETHYLENE GLYCOLS			
<div style="text-align: center;">  </div> <div style="text-align: right;">(I)</div>			
(57) Abstract A pharmaceutical preparation for application of biologically active substance(s) to a mucosal membrane of a mammal comprising one or more substance(s) selected from the group consisting of n-glycofurols represented by formula (I), wherein n is 1 to 8, and n-ethylene glycols represented by the formula (II): H(OCH ₂ CH ₂) _p OH, wherein p is 1 to 14, produces a high plasma concentration of the pharmaceutically active substance(s) nearly as rapid as by i.v. administration.			

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A pharmaceutical preparation containing n-glycofurols
and n-ethylene glycols

The present invention relates to pharmaceutical compositions for administration of a biologically active substance to a mammal via a mucosal membrane.

5 The administration by injection (intravenous, intramuscular and subcutaneous) of biologically active substances is normally regarded as the most convenient way of administration when the purpose is to achieve a rapid and strong systemic effect, e.g. within 3-5 minutes, and when the active substance is not
10 absorbed or is inactivated in the gastrointestinal tract or by first-pass hepatic metabolism. However, the administration by injection presents a range of disadvantages. Thus it requires the use of sterile syringes and may cause pains and irritations, particularly in the case of repeated injections,
15 including the risk of infection. Besides, injections cannot be administered by untrained persons.

Intranasal administration is currently receiving special interest, attempting to avoid the inconveniences caused by the direct invasion into the organism in connection with parenteral
20 administration. Furthermore, this route of administration may conveniently be used as an alternative to parenteral injection, when a rapid onset of effect is needed, and it can be performed by an untrained person.

In order to be an attractive alternative to injection,
25 intranasal administration should offer a similar relation of dosis to plasma concentration and should not cause any considerable pain or irritation to the patient nor any irreversible damage or irritation to the nasal mucosa. However, in the case of treatment of acute health threatening
30 indications, a relatively high local irritation to the mucosa may be acceptable.

In nasal administration, the biologically active substance must be applied to the mucosa in such a condition that it is able to penetrate or be absorbed through the mucosa. In order to penetrate the mucus the vehicle must be biocompatible with the mucus and hence have a certain degree of hydrophilicity. However, the vehicle should preferably also possess lipophilic properties in order to dissolve a physiologically active amount of certain biologically active substances.

The extensive network of blood capillaries under the nasal mucosa is particularly suited to provide a rapid and effective systemic absorption of drugs. Moreover, the nasal epithelial membrane consists of practically a single layer of epithelial cells (pseudostratified epithelium) and it is therefore more suited for drug administration than other mucosal surfaces having squamous epithelial layers, such as the mouth, vagina, etc. These surfaces, however, are also suited for the application of biologically active substances using the drug delivery system according to the invention.

The effective nasal absorption is considered very small if the biologically active substances is not available in water-soluble form (Proctor, 1985). This statement puts severe limitations on the use of the biologically active substances, which should be water soluble and stable in aqueous solutions.

A large number of biologically active substances, including drugs (such as benzodiazepines), vitamins and vaccines, have a limited degree of water-solubility and very often it is not possible to dissolve a clinically relevant amount in the relatively small volume which may be applied for intranasal administration.

For liquid compositions it is essential that an effective amount of the biologically active substance(s) can be dissolved in a volume of less than about 300 μ l. A larger volume can be disagreeable to the patient and will evidently drain out

anteriorly through the nostrils or posteriorly toward the pharynx. The result is that a part of the active substance is lost from the absorption site and that in practice it will be impossible reproducibly to administer a correct dose. The
5 volume for human adults is preferably from about 1 μ l to about 1000 μ l and more preferably from about 50 μ l to about 150 μ l per nostril.

The mucosal epithelium in the nasal cavity is covered with many hair-like cilia being an important defense mechanism for the
10 mammal body against inhaled dust, allergens and micro-organisms. The normal half-time for non-absorbed substances administered to the nasal cavity is about 15 minutes due to the mucociliary clearance removing foreign particles and excess mucus toward the pharynx. For this reason it is preferred that
15 the absorption occurs rapidly and preferably within 1 to 20 minutes.

A variety of vehicle systems for the nasal delivery of biologically active substances has been developed. Up to date the literature has suggested that uptake of biologically active
20 substances from the nasal mucosa may be made possible by incorporation into the formulation of a special vehicle system or by the addition of certain absorption enhancing agents.

Lau and Slattery (1989) studied the absorption characteristics of diazepam and lorazepam following intranasal administration
25 for the treatment of status epilepticus. In order to solubilize these drugs, a non-ionic surfactant, polyoxyethylated castor oil, was selected as the least irritating out of several solvents studied including polyethyleneglycol 400. Diazepam absorption was 84 and 72%, respectively, in two adults measured
30 over a period of 60 hours. However, the peak concentration was not observed until 1.4 hours after the nasal administration and was only about 27% with reference to i.v. administration, which indicate that most of the absorption had probably taken place after the test substance was cleared down to pharynx and

swallowed. Similar results were obtained for lorazepam giving an even longer time to peak (2.3 hours). The authors conclude that the intranasal route of administration had limited potential for the acute treatment of epileptic seizures.

5 Wilton et al. (1988) attempted to administer midazolam to 45 children for achieving preanaesthetic sedation. The volumes used were very impractical and exceeded the maximal volume required for efficient administration resulting in coughing and sneezing with expulsion of part of the dose. The paper does not
10 describe the aqueous vehicle system used.

International Patent Publication No. WO 86/04233 discloses a pharmaceutical composition wherein the drug (e.g. diazepam) is dissolved in a mixture of propellant and co-solvent e.g. glycerolphosphatide. This composition requires a pressurized
15 system and at least one halogenated hydrocarbon aerosol propellant.

Morimoto et al. (1987) have studied a gel preparation for nasal application in rats of nifedipine containing the gelling agent carbopol (polyacrylic acid) in polyethyleneglycol 400 (PEG
20 400), for achieving prolonged action and high bioavailability of the drug. A mixture of equal amounts of carbopol and PEG 400 was preferred. It was shown that nasal application provided higher bioavailability of nifedipine than after peroral administration, but the plasma peak concentration was not
25 observed until after 30 minutes, and it was only $\leq 10\%$ as compared with intravenous administration.

DK patent application no. 2586/87 discloses a pharmaceutical composition comprising an antiinflammatory steroid, water and only 2 to 10 volume-percent propylene glycol, 10 to 25 volume-
30 percent polyethyleneglycol 400 and 1 to 4 volume-percent Tween
20.

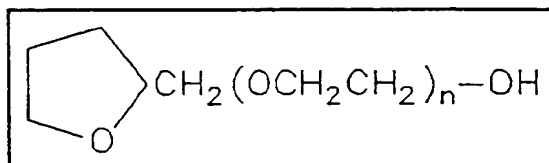
U.S. Patent No. 4,153,689 discloses a principle for obtaining a stable aqueous solution of insulin intended for intranasal administration. It has a pH in the range from 2.5 to 4.7 and it contains from 0.1 to 20 weight percent of a stabilizing agent selected from the group consisting of (a) at least one non-ionic surface active agent with hydrophile-lipophile balance value in the range of 9 to 22, (b) a stabilizing agent selected from polyethylene glycol's having a molecular weight in the range from 200 to 7500 and (c) mixtures of stabilizing agents mentioned in (a) and (b).

International Patent Publication No. 90/02737 discloses nasal administration of benzodiazepine hypnotics in a pharmaceutically acceptable nasal carrier. The carrier may be a saline solution, an alcohol, a glycol, a glycol ether or a mixture thereof. There is no indication that the presence of a glycol or a glycol ether should impart special advantages to the preparation, nor that the presence should be critical for the administration

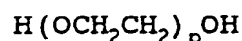
Other preparations for intranasal or sublingual administration are disclosed in US Patent no. 4,746,508 disclosing the uptake of e.g. insulin by using fusidic acid and derivatives as absorption promoters and in International Patent Publication No. WO 87/05210 disclosing a sublingual sprayable pharmaceutical preparation optionally comprising PEG and requiring ethanol, diglyceride and/or triglyceride of fatty acid and a pharmaceutically acceptable propellant gas.

The primary object of the invention is to provide a pharmaceutical composition for application of a biologically active substance via a mucosal membrane of a mammal, which composition is capable of producing a high plasma concentration of the pharmaceutically active substance nearly as rapid as by i.v. administration, without causing unacceptable damage to the mucosal membrane. This object is fulfilled with the composition of the invention.

The pharmaceutical preparation of the present invention is characterized in comprising one or more substance(s) selected from the group consisting of n-glycofurols represented by the formula I:



5 wherein n is an integer of 1 to 8, and n-ethylene glycols represented by the formula II:



wherein p is an integer of 1-14.

According to one aspect of the invention, the n-glycols are
10 such wherein p is 1-8 when no glycofurol is present and when the biologically active substance is a benzodiazepine.

According to a preferred aspect of the invention the n-glycols are such wherein p is 1-8 and according to a more preferred embodiment, the preparation comprises tetraethylene glycol.

15 The pharmaceutical composition of the invention preferably comprises n-glycofurols wherein n is 1 or 2.

In accordance with a preferred aspect of the invention is provided a pharmaceutical preparation comprising one or more n-glycofurols and optionally one or more n-ethylene glycols.

20 The pharmaceutical composition of the invention may comprise a biologically active substance selected from the group consisting of Adrenal hormones, corticosteroids and derivatives such as ACTH and analogues, teracosactrin, alsactide, cortisone, cortisone acetate, hydrocortisone, hydrocortisone
25 alcohol, hydrocortisone acetate, hydrocortisone hemisuccinate, prednisolone, prednisolone terbutate, 9-alpha-fluoroprednisolone, triamcinolone acetonide, dexamethasone

phosphate, flunisolide, budesonide, toxicorol pivalate, etc.;
Amino acids; Anorectics such as benzphetamine HCl,
chlorphentermine HCl, etc.; Antibiotics such as tetracycline
HCl, tyrothricin, cephalosporine, aminoglycosides,
5 streptomycin, gentamycin, leucomycin, penicillin and
derivatives, erythromycin, etc.; Anti-allergic agents;
Antibodies such as monoclonal or polyclonal antibodies against
infectious diseases; Anti-cholinergic agents such as atropine
base, etc.; Anti-depressants such as amitriptyline HCl,
10 imipramine HCl, etc.; Anti-emetica such as neuroleptica, e.g.
metopimazin, antihistamins e.g. thienylperazin or anti-emetica
having a regulatory effect on the motility of the intestine
such as domperidon; Anti-epileptica and anti-spasmolytica such
as clonazepam, diazepam, nitrazepam, lorazepam etc.; Anti-
15 histaminic and histaminic agents such as diphenhydramin HCl,
chloropheniramine maleate, clemastine, histamine,
prophenpyridamine maleate, chlorprophenpyridamine maleate,
disodium cromoglycate, meclizine, etc.; Anti-hypertensive
agents such as clonidine HCl, etc.; Anti-inflammatory agents
20 (enzymatic) such as chymotrypsin, bromelain seratiopeptidase,
etc.; Anti-inflammatory agents (non-steroidal) such as
acetaminophen, aspirin, aminopyrine, phenylbutazone, mefenamic
acid, ibuprofen, diclofenac sodium, indomethacin, colchicine,
probenocid, etc.; Anti-inflammatory agents (steroidal) such as
25 hydrocortisone, prednisone, fluticasone, predonisolone,
triamcinolone, triamcinolone acetonide, dexamethasone,
betamethasone, beclomethasone, beclomethasone dipropionate,
etc.; Anti-neoplastic agents such as actinomycin C, etc.; Anti-
septics such as chlorhexidine HCl, hexylresorcinol, dequalinium
30 chloride, ethacridine, etc.; Anti-tumor agents; Anti-tussive
expectorant (asthmatic agents) such as sodium cromoglycate,
codeine phosphate, isoproterol HCl, etc.; Anti-viral and anti-
cancer agents such as interferons (such as Alpha-2 interferon
for treatment of common colds), phenyl-p-guanidino benzoate,
35 enviroxime, etc.; Beta-adrenergic blocking agents such as
propranolol HCl, etc.; Blood factors such as factor VII, factor
VIII etc.; Bone metabolism controlling agents such as vitamine

D₃, active vitamine D₃, etc.; Bronchodilators such as clenbuterol HCl, bitolterol mesylate, etc.; Cardiotonics such as digitalis, digoxin, etc.; Cardiovascular regulatory hormones, drugs and derivatives such as bradykin antagonists, 5 atrial natriuretic peptide and derivatives, hydralazine, angiotensin II antagonist, nitroglycerine, nifedipine, isosorbide dinitrate, propranolol, clofilium tosylate, etc.; Chemotherapeutic agents such as sulphathiazole, nitrofurazone, etc.; CNS-stimulants such as lidocaine, cocaine, etc.; 10 Corticosteroids such as laticortone, hydrocortisone, fluocinolone acetonide, triamcinolone acetonide, etc.; Diagnostic drugs such as phenolsulfonphthalein, dey T-1824, vital dyes, potassium ferrocyanide, secretin, pentagastrin, cerulein, etc.; Dopaminergic agents such as bromocriptine 15 mesylate. etc.; Enzymes such as lysozyme chloride, dextranase, etc.; Gastrointestinal hormones and derivatives such as secretin, substance P, etc.; Hypothalamus hormones and derivatives such as LHRH and analogues (such as nafarelin, buserelin, zolidex, etc.), enkephalins (DADLE, metkephamid, 20 leucine enkephalin), TRH (thyrotropin releasing hormone), etc.; Hypothensives; Local anesthetics such as benzocain, etc.; Migraine treatment substances such as dihydroergotamine, ergometrine, ergotamine, pizotizin, etc.; Narcotics, antagonists and analgetics such as buprenorphine, naloxone 25 etc.; Pancreatic hormones and derivatives such as insulin (hexameric/dimeric/ monomeric forms), glucagon, etc.; Parasympathomimetics such as nicotine, methacholine, etc.; Parasympatholytics such as scopolamine, atropine, ipratropium, etc.; Parkinson disease substances such as apomorphin etc.; 30 Pituitary gland hormones and derivatives such as growth hormone (e.g. human), vasopressin and analogues (DDAVP, Lypressin), oxytocin and analogues, etc.; Prostaglandines such as PGA and derivatives, PGE₁ and derivatives, PGE₂ and derivatives, PGF₁ and derivatives, dinoprost trometamol, etc.; Protease inhibitors 35 such as aprotinin, citrate or α_1 -antitrypsin etc.; Sedatives such as alprazolam, bromazepam, brotizolam, camazepam, chlordiazepoxide, clobazam, chlorazepic acid, clonazepam,

clotiazepam, cloxazolam, delorazepam, diazepam, estazolam, ethyl loflazepate, fludiazepam, flunitrazepam, flurazepam, flutazolam, halazepam, haloxazolam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, 5 nitrazepam, nordiazepam, oxazepam, oxazolam, pinazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam, etc.; Sex-hormones such as ethinyloestradiol, levonorgestrel, FSH, LH, LTH, estradiol-17-beta, progesterone, norethindrone, testosterone, etc.; Sympathomimetics such as ephedrine, 10 epinephrine, phenylephrine, xylometazoline, tramazoline, dopamine, dobutamine, etc.; Thyroid gland hormones and derivatives such as calcitonins and synthetic modifications thereof etc.; Tranquillisers such as alprazolam, bromazepam, brotizolam, camazepam, chlordiazepoxide, clobazam, chlorazepic 15 acid, clonazepam, clotiazepam, cloxazolam, delorazepam, diazepam, estazolam, ethyl loflazepate, fludiazepam, flunitrazepam, flurazepam, flutazolam, halazepam, haloxazolam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, nitrazepam, nordiazepam, oxazepam, 20 oxazolam, pinazepam, prazepam, temazepam, tetrazepam, tofisopam, triazolam, etc.; Vaccines such as AIDS-vaccines, influenza virus, parainfluenza virus, measles, polio, rhinovirus type 13, respiratory syncytial virus, etc.; Vasoconstrictors such as phenylephrine HCl, tetrahydrozoline 25 HCl, naphazoline nitrate, oxymetazoline HCl, tramazoline HCl, etc.; Vasodilators such as nitroglycerin, papaverine HCl, substance P, VIP (vasoactive intestinal peptide) etc; Vitamines such as vitamin-B₁₂, folic acid, or nicotinamide.

The composition of the invention comprises, according to a 30 preferred aspect of the invention, a biologically active substance selected from the group consisting of Adrenal hormones, Corticosteroids and derivatives thereof, Amino acids, Anorectics, Antibiotics, Anti-allergic agents, Anti-cholinergic agents, Anti-depressants, Anti-dots, Anti-epileptics, Anti- 35 histaminic and histaminic agents, Anti-hypertensiva, Anti-inflammatory agents (enzymatic, non steroidal and steroidal),

Anti-neoplastic agents, Anti-septics, Anti-tumour agents, Anti-tussive expectorants (asthmatic agents), Anti-viral and anti-cancer agents, Beta-adrenergic blocking agents, Blood factors, Bone metabolism controlling agents, Bronchodilators, 5 Cardiotonics, Cardiovascular regulatory hormones, drugs and derivatives, Chemotherapeutic agents, CNS-stimulants, Corticosteroids, Diagnostic drugs, Dopaminergic agents, Enzymes, Fibrinolytics, GABA antagonists, Gastrointestinal hormones and derivatives, Glutamate antagonists, Glycine 10 antagonists, Hypothalamus hormones and derivatives, Hypothensives, Local anaesthetics, Migraine treatment substances, Narcotics, antagonists and analgetics, Pancreatic hormones and derivatives, Parasympathomimetics, Parasympatholytics, Pituitary gland hormones and derivatives, Prostaglandins, 15 Sedatives, Sex-hormones, Spasmolytics, Sympathomimetics, Thyroid gland hormones and derivative, Tranquillises, Vaccines, Vasoconstrictors, Vasodilators, and Vitamins.

According to another aspect of the invention, the biologically active substance(s) is(are) selected from biologically active 20 peptides which are digested in the gastrointestinal tract.

According to a more preferred aspect of the invention, the biologically active substance is selected from the group consisting of coagulation factors such as Factor VII, Factor VIII, Factor IX and derivatives and analogues thereof; agents 25 controlling bone metabolism such as Vitamine D₃, active Vitamine D₃, calcitonin and derivatives and analogues thereof; hormones secreted by hypothalamus such as LHRH or analogues, e.g. nafarelin, buserelin or Zolidex, enkephalins such as DADLE, metkephamid or leucine enkephalin, and TRH and derivatives and 30 analogues thereof; hormones secreted by pancreas such as insulin or glucagon and derivatives and analogues thereof; hormones secreted by the pituitary gland such as growth hormone, vasopressin or oxytocin and derivatives and analogues thereof, e.g. DDAVP or lypressin; sex-hormones such as 35 ethinylestradiol, levonorgestrol, FSH, LH, LTH estradiol-17 β ,

progesterone, norethindrone or testosterone and derivatives and analogues thereof; and tranquilizer such as alprazolam, bromazepam, brotizolam, camazepam, chlordiazepeoxide, clobazam, chlorazepic acid, clonazepam, clotiazepam, cloxazolam, 5 delorazepam, diazepam, estazolam, ethyl loflazepate, fludiazepam, flunitrazepam, flurazepam, flutazolam, halazepam, haloxazolam, ketazolam, loprazolam, lorazepam, lormetazepam, medazepam, midazolam, nimetazepam, nitrazepam, nordiazepam, oxazepam, oxazolam, pinazepam, prazepam, temazepam, tetrazepam, 10 tofisopam or triazolam and salts, derivatives and analogues thereof.

According to another more preferred aspect of the invention, the biologically active substance selected from the group consisting of coagulation factors such as Factor VII, Factor 15 VIII, Factor IX and derivatives and analogues thereof; agents controlling bone metabolism such as calcitonin and derivatives and analogues thereof; hormones secreted by hypothalamus such as LHRH or analogues, e.g. nafarelin, buserelin or Zolidex, enkephalins such as DADLE, metkephamid or leucine enkephalin; 20 hormones secreted by pancreas such as insulin or glucagon and derivatives and analogues thereof; hormones secreted by the pituitary gland such as growth hormone, vasopressin or oxytocin and derivatives and analogues thereof, e.g. DDAVP or lyspressin; sex-hormones such as FSH, LH or LTH and derivatives and 25 analogues thereof.

The present invention is of particular importance when administering active substances being biologically active peptides which are digested in the gastrointestinal tract such as insulin, glucagon, growth hormone or insulin like growth 30 factors or derivatives or analogues thereof.

In accordance with a more preferred aspect of the invention, the active substance is a pancreatic polypeptide hormone such as insulin and glucagon or a derivative or an analogue thereof.

In accordance with another preferred aspect of the invention, the active substance is selected among antiepileptics, spasmolytics and tranquillisers selected from the group of benzodiazepines such as clonazepam, diazepam, flunitrazepam, 5 triazolam, lorazepam, nitrazepam or mixtures thereof.

The active substance may be present in an amount of from 0.0001% to 50% of the total composition, preferably in an amount of from 0.001% to 20% e.g. in the case of benzodiazepines.

- 10 The pharmaceutical preparation of the invention may furthermore comprise nitric acid and/or nitrate in a concentration ranging from 0.0001 to 5%. Such component may act as a stabilizer for active substances such as clonazepam.

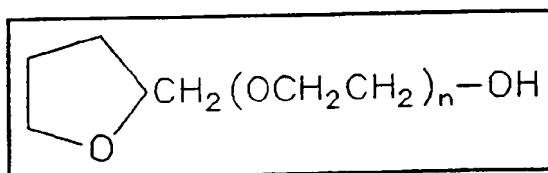
According to a further aspect of the invention the 15 pharmaceutical preparation additionally comprises one or more compound(s) selected from the group consisting of surfactants, absorption promoters, water absorbing polymers, microspheres, oils, emulsions, liposomes, substances that inhibit enzymatic degradation, alcohols, organic solvents, water, hydrophobic 20 agents, pH-controlling agents, preservatives and osmotic pressure controlling agents, cyclodextrines and propellants or mixtures thereof.

Preferably, the water absorbing polymers are polyethylene glycols having an average molecular weight ranging from 200 to 25 7500 or propylene glycol or mixtures thereof.

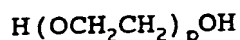
According to a preferred aspect of the invention the composition comprises more than 50% (w/w) of polyethylene glycol having an average molecular weight ranging from 200 to 1000 and/or propylene glycol or mixtures thereof.

- 30 The invention also relates to a method for treatment of a mammal with a biologically active substance wherein the

biologically active substance is applied via a mucosal membrane of the mammal to be treated together with one or more substance(s) selected from the group consisting of n-glycofurols represented by the formula I:

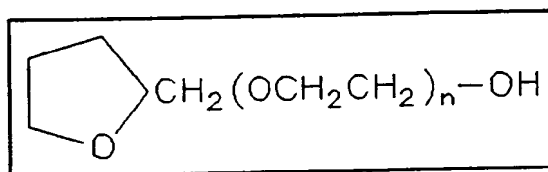


5 wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:



wherein p is 1 to 14.

According to a further aspect the invention relates to the use
10 of a pharmaceutical composition comprising a biologically active substance and one or more substance(s) selected from group consisting of n-glycofurols represented by the formula I:



wherein n is 1 to 8, and n-ethylene glycols represented by the
formula II:



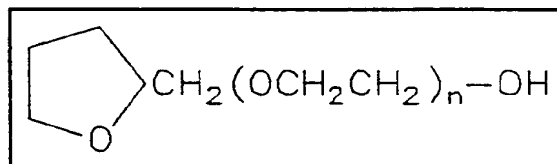
wherein p is 1 to 14 for application to a mucosal membrane.

The mucosal membrane to which the pharmaceutical preparation of the invention is administered may be any mucosal membrane of the mammal to which the biologically active substance is to be
20 given, e.g. in the nose, vagina, eye, mouth, genital tract, lungs, gastrointestinal tract, or rectum, preferably the mucosa of the nose, mouth or vagina.

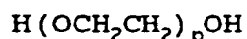
The pharmaceutical compositions of the invention may be administered in the form of a sublingual lozenge or troche or a buccal or nasal spray in the form of a solution, a suspension or a powder, optionally together with various absorption enhancers or more preferred in the form of slightly viscous solution which is bioadhesive or optionally in the form of a suppository or vagitory.

According to a preferred aspect of the invention, the application is to the nasal mucosa.

- 10 The invention relates, according to a still further aspect, a method for producing a pharmaceutical composition for application of a biologically active substance via a mucosal membrane of a mammal comprising one or more substance(s) selected from group consisting of n-glycofurols represented by
15 the formula I:



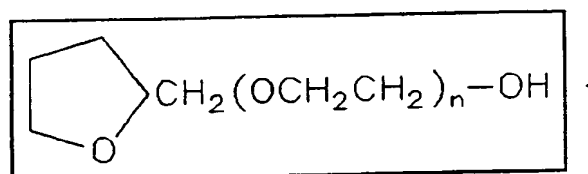
wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:



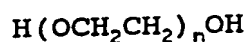
- wherein p is 1 to 14, wherein the biologically active substance
20 is dissolved in a prepared vehicle comprising a part of or all the remaining constituents of the pharmaceutical preparation. The method may be carried out using ultrasound and/or at an elevated temperature. It is preferred to carry out the dissolution during heating to a temperature of from 30°C to
25 100°C taking into account the temperature sensitivity of the active substance. For very sensitive active substances such as human growth hormone only mild stirring or shaking is advisable.

It is another aspect of the invention to provide a controlled release delivery system for intranasal application comprising even substantially non-aqueous vehicles, which are biocompatible with the mucus and which are capable of dissolving required amounts of biologically active substances in small volumes.

According to get another aspect the invention relates to the use of one or more substance(s) selected from the group consisting of n-glycofurols represented by the formule I:



wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:



wherein p is 1 to 14 for the preparation of a pharmaceutical preparation for application of a biologically active substance to a mammal through a mucosal membrane.

The use according to this aspect of the invention is preferably for the preparation of pharmaceutical preparations for systemic administration through the mucosa of the nose, mouth or vagina, most preferred through the mucosa of the nose.

According to one aspect of the invention n-glycofurols of the formula I are considered as a pharmaceutical acceptable carrier, especially a pharmaceutical acceptable nasal carrier. According to another aspect of the invention n-glycofurols of the formula I are considered as an enhancer facilitating the uptake of a biologically active substance through a mucosal membrane of a mammal, especially through the mucosa of the nose.

The present invention renders it possible to normalize e.g. the blood glucose level in subjects suffering from abnormalities in their sugar metabolism such as hypoglycaemia or hyperglycaemia by giving glucagon or insulin or an analogue or derivative thereof respectively, in a very easy and convenient manner.

Furthermore, the invention enables very convenient administration of bone metabolism controlling agents such as vitamin D₃ or Calcitonin and derivatives and analogues thereof; hormones secreted by hypothalamus such as LHRH such as nefarelin, buserelin or Zolidex and derivatives and analogues thereof; hormones regulating the growth or mitogenic growth factors such as somatropin, IGF-1, IGF-2, FGF, PDGF, TGF, EGF, and derivatives and analogues thereof; protease inhibitors such as aprotinin, citrate or α_1 -antitrypsin derivatives and analogues thereof; and cytochines such as IL-1 and IL-2, in order to normalize the blood level thereof in case of a deficiency.

Especially preferred for use in vehicle compositions according to the invention is glycofurol 75 (GF) which refers to commercially available solvents of polymers of the above formula I, wherein n is mainly 1 and 2. (Chemical Abstract Registration No. [9004 76-6]). Glycofurol 75 is a colourless liquid miscible with water, alcohols, such as methanol, ethanol, n-propanol, glycerol and various oils in all proportions and has a b.p. about 155°C. GF is reported to cause irritation when used in compositions for parenteral administration undiluted form as reviewed by Spiegel and Nosewothy (1963). It has been reported to be non-toxic and non-irritating when diluted in water (Spiegelberg et al., 1956).

The n-ethylene glycols used in accordance with the present invention may e.g. be monoethylene glycol (1EG), diethylene glycol (2EG), triethylene glycol (3EG), tetraethylene glycol (4EG), pentaethylene glycol (5EG), hexaethylene glycol (6EG), heptaethylene glycol (7EG), octaethylene glycol (8EG),

nonaethylene glycol (9EG), decaethylene glycol (10EG), undecaethylene glycol (11EG), dodecaethylene glycol (12EG), tridecaethylene glycol (13EG) and tetradecaethylene glycol (17EG). The ethylene glycols may be used in the form of the
 5 single compounds or a mixture of two or more n-ethylene glycols, e.g. commercial products such as polyethylene glycol 200 (PEG 200) or polyethylene glycol 400 (PEG 400).

1EG to 14EG are colourless liquids miscible with water and alcohols in all proportions. PEG 200 is a commercially
 10 available mixture of ethylene glycols having an average molecular weight of about 200. The composition is shown in Table 1:

Table 1: Composition of PEG 200

15	Monoethylene glycol	0.1%
	Diethylene glycol	3.4%
	Triethylene glycol	21.2%
	Tetraethylene glycol	31.2%
	Pentaethylene glycol	24.4%
20	Hexaethylene glycol	14.0%
	Heptaethylene glycol	5.4%
	Octaethylene glycol	0.3%
		<hr/> 100.0%

NEG's and glycofuroles are available in various qualities. Especially preferred are highly purified qualities such as 4EG
 25 from Fluka-Chemie AG (Buchs, Switzerland, art. no.: 86660, 1990), abbreviated 4EGf.

The delivery system according to the invention can be optimized e.g. with respect to bioadhesion, sprayability or viscosity. E.g. GF, in concentration of only 5%, has a surprisingly
 30 positive effect on the sprayability of e.g. 4EGf by reducing viscosity. Furthermore, the addition of 5% of GF is able to decrease the solidification temperature from about -10 to \leq -20°C. This is of importance where the formulation should be carried on persons or transported outdoor, ready for use.

The pharmaceutical composition of the invention may be used to treat animals such as domestic animals or pets or, preferably, human beings.

A special advantage in using the above vehicle system is that 5 e.g. highly lipophilic substances such as the benzodiazepines as well as water soluble substances e.g. peptides and proteins such as the pancreatic hormones can be solubilized in clinically relevant dose for human subjects in only e.g. 25-300 μ l of the vehicle. In aqueous solutions clinically relevant 10 doses of diazepam and clonazepam will alternatively have to be dissolved in about 5000 ml and >10 ml, respectively.

The vehicle system according to the invention may be used in combination with various co-solvents, such as vegetable oil such as Miglyol® 840 (Dynamit Nobel Chemie, Troisdorf, W-15 Germany) or optionally hydrogenated or ethoxylated castor oil, which surprisingly increases the possibilities for designing a controlled release - formulation such as a diazepam formulation which avoids peak plasma concentrations.

The composition according to the invention may comprise one or 20 more additional pharmaceutical exipients, such as: surfactants and absorption promoters having a hydrophilic-lipophilic balance from about 6 to 26 and ionic as well as non-ionic surfactants including polyoxyethylene alcohol ethers, bile salts and derivatives thereof, fusidic acid and derivatives 25 thereof, oleic acid, lecithin, lysolecitins, or Tween 20 to 85; Water absorbing polymers, such as polyethyleneglycol 200 to 7500, polyvinylpyrrolidone, propyleneglycol, or polyacrylic acid, gelatine, cellulose and derivatives; Substances which inhibit enzymatic degradation, such as, citrate or aprotinin; 30 Alcohols, such as, ethanol, glycerol, or benzylalcohol; Organic solvents, such as, ethylacetate, or benzylalcohol; Hydrophobic agents, such as vegetable oil, e.g. soybean oil, peanut oil, coconut oil, corn oil, olive oil, sunflower oil, castor oil, Miglyol® 810/812/840 or mixtures thereof; pH-controlling

agents, such as, nitric acid, phosphoric acid, or acetic acid, citrate; Preservatives and osmotic pressure controlling agents, such as glycerol, sodium chloride, methyl paraoxybenzoate, or benzoic acid; Powder compositions, such as, alfa-, beta- and
5 gamma-cyclodextrines, cellulose and derivatives; Microspheres, liposomes and emulsions compositions, such as, starch, albumin, gelatine, or lecithins or lysolecithins; Microencapsulated formulations; Propellants such as butane; Water. The use of alcohols or propellants are not mandatory in the composition
10 according to the invention.

The invention is further illustrated with reference to the accompanying drawings in which

Fig. 1 shows a graphical representation of the mean plasma concentration of Clonazepam after intravenous
15 administration and intranasal administration of a preparation in accordance with the invention,

Fig. 2 shows a block diagram of the mean time to response as a function of the contents of glycofurol in a preparation according to the invention,

20 Fig. 3 shows a graphical representation of the blood glucose level after administration of insulin in accordance with the invention,

Fig. 4 shows a graphical representation of the blood glucose level after administration of glucagon in accordance
25 with the invention,

Fig. 5 shows a graphical representation of the concentration of Clonazepam in plasma after administration of a preparation comprising various ethylene glycol constituents,

- Fig. 6 shows a graphical representation showing the mean plasma level of diazepam after administration of preparations comprising glycofurol and various co-solvents,
- 5 Fig. 7 shows a graphical representation of the effect of various contents of glycofurol in preparations according to the invention,
- Fig. 8 shows a graphical representation comparing the plasma level of flunitrazepam after i.v. administration and
10 intranasal administration according to the invention,
- Fig. 9 shows a graphical representation comparing the plasma level of clonazepam after i.v. administration and intranasal administration according to the invention,
- Fig. 10 shows a diagram showing the stability of clonazepam
15 in various vehicles,
- Fig. 11 shows a graphical representation comparing the plasma concentrations of estrogen after i.v. administration and intranasal administration according to the invention, and
- 20 Fig. 12 shows a graphical representation comparing the plasma concentrations of estrone after i.v. administration and intranasal administration of estrogen according to the invention.

The invention is explained more in detail with reference to the
25 Examples which are to be considered only as illustrating the invention and not to be construed so as to limit the scope of the invention as set forth in the appended claims.

Example 1Toxicity and Acceptability study of the Vehicles according to the invention.

In a toxicological evaluation of the vehicle the local as well as the systemic effect after absorption should be considered. GF and PEG 400 have been used as excipient in injection formulations, where the administered amount is greater than 300 μ l per dose, which exceeds the amount administered intranasally using the compositions according to the invention.

- 10 Local toxicity related results for nEG and GF after intranasal administration are not available from the literature. Therefore, tests were conducted assessing the local toxicity of 30 - 100 μ l to rabbit nasal mucosa. In these tests benzodiazepines were also dissolved in various solvent systems according to the invention and 30 μ l of the composition was applied daily for 14 days to the nasal mucosa of rabbits. The effect of the thus administered compositions was then assessed in five experiments including a control carried out as stated in Table 2:

Table 2

Assessment of local toxicity of benzodiazepine compositions according to the invention.

5	Animal No/Sex	Left Nostril		
		Group	Test Material	Session/Day
10	1 m 2 3 4 f 5 6	1 Control	30 μ l Saline	1
15	7 m 8 9 10 f 11 12	2	30 μ l D in PEG	1
25	13 m 14 15 16 f 17 18	3	30 μ l L in PEG	1
30	19 m 20 21 22 f 23 24	4	30 μ l F in PEG	1
35	25 m 26 27 28 f 29 30	5	30 μ l F in PEG + GF	1

Abbreviations:

45 D=Diazepam 3%; L=Lorazepam 5%; F= Flunitrazepam 1%;
PEG=Polyethylene glycol 200; GF=Glycofurol 75; PEG+GF=70% PEG
+ 30% GF.

Table 2 (continued)

Assessment of local toxicity of vehicles of compositions according to the invention.

5	Animal No/Sex	Right Nostril		
		Group	Test Material	Session/Day
10	1 m 2 3 4 f 5 6	A Control	100 μ l Saline	3
15	7 m 8 9	B C D	30 μ l PEG 100 μ l PEG 100 μ l PEG	1 1 3
20	10 f 11 12	E F G	30 μ l PEG + GF 100 μ l PEG + GF 100 μ l PEG + GF	1 1 3
25	13 m 14 15 16 f 17 18	B C D E F G	30 μ l PEG 100 μ l PEG 100 μ l PEG 30 μ l PEG + GF 100 μ l PEG + GF 100 μ l PEG + GF	1 1 3 1 1 3
30	19 m 20 21 22 f 23 24	E F G B C D	30 μ l PEG + GF 100 μ l PEG + GF 100 μ l PEG + GF 30 μ l PEG 100 μ l PEG 100 μ l PEG	1 1 3 1 1 3
35	25 m 26 27 28 f 29 30	E F G B C D	30 μ l PEG + GF 100 μ l PEG + GF 100 μ l PEG + GF 30 μ l PEG 100 μ l PEG 100 μ l PEG	1 1 3 1 1 3

Abbreviations:

45 A=Control; PEG=Polyethylene glycol 200; GF=Glycofurol 75;
PEG+GF=70% PEG + 30% GF.

The nasal cavity was examined histologically and only mild inflammatory changes were found after the administration of the

compositions. No clear dose related response was found with increasing doses and no difference between 100% PEG-200 and 70% PEG-200 + 30% GF was found. It was concluded that the vehicles according to the invention will only incur mild reversible
5 toxicological effects.

The tolerance to two test vehicles containing tetraethylene glycol (4EGf) and optionally 5% glycofurol (GF) was studied after intranasal application in nine healthy volunteers, and compared, after a double blind three way cross-over design,
10 with the vehicle of a marketed product, Locilan® (Astra-Syntex Scandinavia AB, Södertälje, Sweden). As control saline (0.9% sodium chloride) was used.

Saline and the vehicle of the marketed product were very well tolerated. The two vehicles containing tetraethylene glycol
15 caused burning sensation or stinging and runny nose of moderate severity immediately after application. The symptoms were short lasting, being over or only of mild severity 10 min. after application. No clinical symptoms could be observed by nasoscopy 30 minutes after the application.

20 When questioned all subjects stated, that the 4EGf-containing vehicles optionally comprising 5% GF were acceptable for administration of essential biologically active substances to be used occasionally, and that they would prefer intranasal application in one of these vehicles to intravenous
25 administration.

The experimental details and results are given below.

Acceptability study of the vehicles according to the invention in human subject.

Subjects

Nine healthy volunteers were enrolled in the trial. All 5 subjects were healthy without any cardiac, hepatic or nephrological diseases.

None of the subjects were suffering from any pulmonary or respiratory diseases or had had a cold within the last 2 weeks. Vital signs (blood pressure and pulse) were within normal range 10 for all subjects. Standard tests for biochemistry and haematology were carried out within two weeks prior to the study. In all but two subjects, all clinical chemical data were within normal range. In subject nos. 4 and 5 alanine aminotransferase (SPGT) was above upper normal limit, but this 15 was considered without clinical significance.

Study procedure

The study was a double blind test of three test vehicles and a control (saline) and consisted of three identical sessions, with 24 hours between each session.

20 During each session a control vehicle was administered into the right nostril 5 minutes prior to application of a test vehicle into the left nostril. The subjects were asked to record in a questionnaire any pain or irritation that was observed 0-1 min. after application, and 10 and 30 minutes after application of 25 the test vehicle. If any symptoms were noted, the subject were to record the severity and to characterize the symptom(s).

Prior to application of vehicles and 30 minutes after application of the test vehicle the mucous membrane in both nostrils were inspected by the experimenter by nasoscopy.

Vehicles

Four vehicles were tested, one being saline and used as a negative control. The other three vehicles were two test vehicles according to the invention (Vehicle A and B) and a 5 "positive control", which is the vehicle of a very well tolerated marketed product, Locilan® (Nielsen et al., 1989).

Devices

A Pfeiffer pump (model 6917.5902/3790.0129) unit delivering 50 μ l when activated was used for application.

10 Dosage

The vehicles were applied after placing the spray unit of the Pfeiffer pump in the left (or right) nostril and activating the pump twice, the total dose being 100 μ l.

Clinical inspection

15 The clinical inspection of the tissue in the nose did not disclose any clinical significant effect of the applicated vehicles. In three of the subjects a mild degree of local irritation was noted 30 minutes after application of the vehicles; the vehicles applied being saline in one subject and 20 test vehicle B in the other two subjects.

Tolerance as reported by the subjects (Table 3-5)

The severity of irritation (symptoms) in the nose 0-1 min., 10 min. and 30 min. after application of test vehicles is summarized in table 3, 4, and 5, respectively.

The subjects concluded that the tetraethyleneglycol vehicles optionally comprising glycofurol were less well tolerated than saline of the vehicle in Locilan®. However, the symptoms caused by the 4EGf-containing vehicles were relatively short lasting and considered acceptable, if the biologically active substances were essential for the individual's health.

Table 3

Tolerance 0-1 min. after application as reported in questionnaires by the subjects: No. of subjects having symptoms and severity thereof.

Severity of irritation	Control (right nostril)	Vehicle		
		A	B	C
15				
No irritation	25	1	0	6
Mild- acceptable	2	5	3	3
Moderate severe	0	3	6	0
20 Unacceptable	0	0	0	0

A = 4EGf - vehicle

B = 5% GF in 4EGf

C = Locilan® vehicle

25 Control = (right nostril) saline.

Table 4:

Tolerance 10 min. after application as reported in questionnaires by the subjects: No. of subjects having symptoms and severity thereof.

Severity of irritation	Control (right nostril)	Vehicle		
		A	B	C
10 No irritation	27	5	5	7
Mild/ acceptable	0	4	4	2
Moderate severe	0	0	0	0
Unacceptable	0	0	0	0

A = 4EGf - vehicle

B = 5% GF in 4EGf

C = Locilan® vehicle

Control = (right nostril) saline.

Table 5:

Tolerance 30 min. after application as reported in questionnaires by the subjects: No. of subjects having symptoms and severity thereof.

5	Severity of irritation	Control (right nostril)	Vehicle		
			A	B	C
10	No irritation	27	8	7	8
	Mild-acceptable	0	1	2	1
	Moderate severe	0	0	0	0
	Unacceptable	0	0	0	0
15					

A = 4EGf - vehicle

B = 5% GF in 4EGf

C = Locilan® vehicle

Control = (right nostril) saline.

20 Vehicle A:

4 EGf-vehicle

1	Tetraethylene glycol puriss p.a.	25 ml
	Concentrated nitric acid p.a.	1drop
11	Tetraethylene glycol puriss p.a.	25 ml
	Solution I (app. 30 drops)ad pH	3.8
25		

The mixture I is used to adjust the pH of II employing a pH-electrode for viscous liquids (Radiometer GK 2711). 2-10 ml of II (vehicle A) is dispensed in a Pfeiffer multidispenser (Pfeiffer Zerstäuber, 6917.5902/3790.0129).

5 Vehicle B:

5% GF in 4EGf-vehicle

I	Tetraethylene glycol puriss p.a.	25 ml
	Concentrated nitric acid p.a.	1 drop
II	Tetraethylene glycol puriss p.a.	25 ml
10	Solution I (app. 30 drops)	ad pH 3.8
III	Solution II	23.75 ml
	Glycofurol 75 purisès	1.25 ml

The mixture I is used to adjust the pH of II using a pH-electrode for viscous liquids (Radiometer GK 2711). Then the 15 mixture III (vehicle B) is prepared. 2-10 ml of the vehicle is dispensed in a Pfeiffer multidispenser (Pfeiffer Zerstäuber; 6917.5902/3790.0129).

Vehicle C:

Lokilan®-vehicle	
Propylene glycol, Ph.Eur. III	2 g
Polyethylene glycol 400, Ph.Nord. 63 II	20 g
5 Tween 20, Ph.Eur. III	2.5 g
Benzalconium chloride, DLS 86	70 mg
Disodium-EDTA, Ph.Eur. 2nd Ed. 1983	10 mg
Butylated hydroxytoluene, Ph.Nord. 63 II, Add.	10 mg
Citric acid, Ph.Eur. III, Suppl. 77	5 mg
10 Sodium citrate, 2H ₂ O, Ph.Eur. III	7.65 mg
Sorbitol DAK 63	2.86 g
Purified water, Ph.Eur. 2nd Ed. 1981	ad 100 g

Example 2

10 mg clonazepam was dissolved in 2 ml of vehicle B (example 1)
 15 using ultrasound to obtain a clonazepam concentration of 5
 mg/ml. 50 µl of this preparation was administered into each
 nasal cavity of male New Zealand White rabbits, held in a
 supine position during and 1 minute after the application. An
 Eppendorph pipette was used for the application. Blood samples
 20 were obtained from the marginal ear vein at 0, 2, 5, 10, 15,
 30 and 60 minutes and the clonazepam concentration was determi-
 ned by HPLC.

Figure 1 shows the mean plasma clonazepam concentration
 obtained after the administration. The figure also shows the
 25 plasma concentration after i.v. injection into the marginal ear
 vein of the same dose (0.5 mg) of clonazepam as Rivotril®,
 injected over ½ minute. The figure shows that the plasma
 concentration after intranasal application is about the same or
 even higher, at about 2 minutes, than for an i.v. injection.

Example 3Pharmacodynamic response, enhanced by GF

The pharmacodynamic response was tested in New Zealand White rabbits after application of 50 μ l of 2.5 mg clonazepam/ml vehicle into each nostril while they were in sitting position. The vehicle consisted of 0, 10, 20, 30, 70 and 100% glycofuro-lum 75 (Roche, Lot no. 706127) added to tetraethylene glycol (Merck, Art. 808619). Clonazepam was dissolved by ultrasonifi-cation.

10 Figure 2 shows the mean time to response (rabbit can stay in a lying position with its hind legs stretched to one side). Number on the top of bars denote the amount of responders out of four tested. Non responders within 10 minutes are calculated as 10 minutes in the mean value. For intranasal administration 15 of clonazepam about 10% GF in 4EGm has an optimal response, whereas about 30% GF has a minimal response. The same pattern is observed for 30% GF in 4EGf.

Example 4

0.33 mg zinc-free (monomeric) human insulin in 50 μ l 12.5 mM 20 phosphate buffer (pH 7.4) containing 5% glycofuro-l 75 was applied into one nostril of 5 sitting New Zealand White rabbits, weighing about 3 kg. Blood samples of 50 μ l were withdrawn from the marginal ear vein after 0, 15, 30, 60 and 120 minutes, and blood glucose level was determined.

25 Figure 3 shows the mean blood glucose level as percentage of initial for the formulation and for 0.9% sodium chloride control. 100 μ l of the same formulation, but without insulin, was administered to the nasal cavity of healthy volunteers.

This formulation could not be distinguished from 0.9% sodium chloride with respect to local irritation.

Example 5

4 mg human glucagon in 100 μ l phosphate buffer (pH 4) containing 5% GF was applied into both nostrils (50 μ l into each) of 5 sitting hypoglycaemic New Zealand White rabbits, weighing about 3 kg. The formulation was prepared by dissolving glucagon in a 7 mM phosphate buffer (pH 2.5), adjusting the pH to 4.0 with 0.1 N sodium hydroxide and finally 5% glycofuroolum 75 was 10 added. The rabbits were made hypoglycaemic by subcutaneous injection of 83 μ g insulin one hour prior to the experiment. Blood samples were continuously withdrawn from the marginal ear vein for blood glucose determination.

Figure 4 shows the mean blood glucose level as percentage of 15 initial after the intranasal application of glucagon.

Example 6

Control of peak value, Pharmacokinetics

10 mg clonazepam was dissolved in 2 ml of following vehicles: (1) 4EGf, (2) PEG-200 and (3) 4EGm (from MERCK-Schuchardt) 20 using ultrasound. 50 μ l of this preparation was administered into each nasal cavity of sitting New Zealand White rabbits by means of an Eppendorph pipette. Blood samples were obtained from the marginal ear vein at following time intervals: 0, 2, 5, 10, 15, 30 and 60 minutes and the clonazepam concentration 25 was determined by HPLC.

Figure 5 shows the mean plasma clonazepam concentration obtained after the intranasal administration. At about 2

minutes the plasma concentration is higher for the 4EGf than for the PEG-200 and 4EGm formulations.

Example 7

Control of peak value, Pharmacokinetics

5 3 mg diazepam in 100 μ l vehicle was prepared and applied to rabbits in a manner analogous to that described in example 6. The following vehicles were used: (1) Glycofurolum 75 (GF); (2) Miglyol 840+GF (7+3) and (3) Vegetable oil+GF (7+3). Blood samples were obtained from the marginal ear vein at following
10 time intervals: 0, 5, 10, 15, 30 and 60 minutes and the diazepam concentration was determined by HPLC.

Figure 6 shows the mean plasma diazepam concentrations obtained after the intranasal administration. An initial peak plasma concentration can be controlled dependent on the GF/oil vehicle
15 used. The plasma concentration for the GF formulation at 5 minutes is about 55% of an intravenous injection of 3 mg diazepam as Stesolid® (Dumex A/S, Denmark).

Example 8

Control of peak value, the role of GF as an enhancer

20 0.5 mg clonazepam in 100 μ l vehicle was prepared and applied to rabbits in a manner analogous to that described in example 6. The following vehicles were used: (1) tetraethylene glycol (Merck) (4EGm); (2) 2% glycofurol 75 (GF) in 4EGm; (3) 5% GF in 4EGm; (4) 10% GF in 4EGm; (5) 30% GF in 4EGm and (6) GF. Blood
25 samples were obtained from the marginal ear vein at following time intervals: 0, 5, 10, 15, 30 and 60 minutes and the clonazepam concentration was determined by HPLC.

Figure 7 shows the mean plasma clonazepam concentrations obtained after the intranasal administration. An initial peak plasma concentration can be controlled dependent on the GF/4EGm ratio in the formulation.

5 Example 9

0.5 mg flunitrazepam in 100 μ l vehicle was prepared and applied to rabbits in a manner analogous to that described in example 6. The following vehicle was used: Polyethylene glycol 200 (Merck Art. 807483). Blood samples were obtained from the 10 marginal ear vein at following time intervals: 0, 5, 10, 15, 30 and 60 minutes and the flunitrazepam concentration was determined by HPLC.

Figure 8 shows the mean plasma flunitrazepam concentrations obtained after intranasal and intravenous (Rohypnol®, Roche) 15 administration of 0.5 mg flunitrazepam.

Example 10

4 mg midazolam in 100 μ l vehicle was prepared and applied to rabbits in a manner analogous to that described in example 6. The following vehicle was used: Polyethylene glycol 200 (Merck, 20 Art. 807483). Blood samples were obtained from the marginal ear vein at following time intervals: 0, 5, 10, 15, 30 and 60 minutes and the midazolam concentration was determined by HPLC.

Figure 9 shows the mean plasma midazolam concentrations obtained after intranasal and intravenous (Dormicum®, Roche) 25 administration of 4 mg midazolam.

Example 11Stability studies

In an attempt to optimize the stability of clonazepam in the vehicles according to the invention, an accelerated study was performed. The stability of clonazepam was studied over one month period at 25 and 60°C, where recovery of about 90% or more at 60°C was considered as very satisfying.

Following formulations were prepared, containing 5 mg/ml clonazepam, except for formulation (1) containing 20 mg/ml; (1) and (2) in 4EGf; (3) as (2) adjusted to pH 3.5 with phosphoric acid; (4) as (2) adjusted to pH 3.5 with citric acid; (5) as (2) added ethanol 9%; (6) as (2) adjusted to pH 3.5 with nitric acid (less than 0.01%); (7) as (2) added water 1%; (8) propylene glycol (PG); (9) as (8) adjusted to pH 3.8 with 0.04% acetic acid; (10) as (2) added acetic acid 0.4%; (11) 4EGf+5%GF added acetic acid 0.4% (pH about 6); (12) as (11) added nitric acid to pH 2. (13) as (11) added benzyl alcohol 3%; (14) as (10) added ethanol 16% and benzyl alcohol 3%; (15) 4EGf+5%GF adjusted to pH 3.5 with nitric acid (less than 0.01%); (16) as (15) added sodium nitrate 0.04%; (17) as (15) added ethanol 5%; (18) 4EGf+5%GF adjusted to pH 4.2 citric acid (0.1%); (19) as (15) added benzyl alcohol 2%; (20) 4EGf+5%GF added sodium nitrate 0.04%.

After 2 and 4 weeks 100 µl samples were withdrawn from the containers and analyzed quantitatively by means of HPLC. Sample concentration (and recovery) was calculated on the basis of the peak height relative to an external clonazepam standard.

As seen in table 7, only nitric acid, nitrate, ethanol and benzyl alcohol increases the stability. The content of water in 4EGf, GF and PG was about 0.05%. Amazingly the stability in e.g. 4EGf was not decreased by addition of 1% water. Further, the stability at 25°C was the same for formulations stored in

ampoules (under N₂) and in 10 ml Pfeiffer pump (model 6917.5902- /3790.0129).

Figure 10 is a schematic diagram over the stability of clonazepam in the various vehicles.

5

Table 7

STABILITY OF CLONAZEPAM AT 25°C AND 60°C IN VARIOUS VEHICLES.
% RECOVERY AFTER 2 AND 4 WEEKS

10	No. Form.	2 WEEKS			4 WEEKS		
		Amp. (-air)	Spraybott.	25°C	Amp. (-air)	Spraybott.	25°C
		25°C	60°C	25°C	25°C	60°C	25°C
	1. 4EGf 20mg/ml	98	91	100	82	73	87
15	2. 4EGf 5mg/ml	98	82	96	83	61	84
	3. 4EGf pH 3.5 (Phosphate)	98	79	100	84	62	85
20	4. 4EGf pH 3.5 (Oxalic acid)	95	43	95	81	18	82
	5. 4EGf Ethanol 9%	100	90	99	85	71	90
25	6. 4EGf pH 3.8 (Nitric acid)	98	88	97	95	87	95
	7. 4EGf Water 1%	94	83	94	89	71	94
30	8. PG	96	69	99	96	56	92
	9. PG pH 3.8 (Acetic acid)	100	99	103	93	95	97
35	10. 4EGf + 0.4% (Acetic acid)	102	84	102	99	67	102

40

Concentration: Formulation No. 1 = ca. 20 mg C/ml
Formulation No. 2-10 = ca. 5 mg C/ml

Table 7 (continued)

STABILITY OF CLONAZEPAM AT 25°C AND 60°C IN VARIOUS VEHICLES
% RECOVERY AFTER 2 AND 4 WEEKS

5	No. Form.	2 WEEKS			4 WEEKS		
		Amp. (-air)	Spraybott.		Amp. (-air)	Spraybott.	
		25°C	60°C	25°C	25°C	60°C	25°C
10	11.4EGf+5%GF Acetic acid 0.4%	101	80	100	98	63	99
15	12.=No.11 pH 2 (Nitric acid)	98	86	no data	97	81	no data
20	13.=No.11 + Benzyl-OH 3%	103	85	102	99	76	96
25	14.=No.10 + Ethanol 16% Benzyl-OH 3%	103	92	no data	100	80	no data
30	15.4EGf+5%GF pH 3.8 (Nitric acid)	99	94	96	96	96	98
35	16.=No.15 + Nitrate 0.04%	102	95	100	96	93	98
40	17.=No.15 + Ethanol 5%	96	98	99	98	87	98
	18.4EGf+5%GF pH 4.2 (Citric acid)	95	85	94	93	67	98
	19.=No.15 + Benzyl-OH 2%	101	96	97	98	93	99
	20.4EGf+5%GF + Nitrate 0.04%	93	75	99	99	78	95

Concentration: Formulation No. 11-20 = ca. 5 mg C/ml

Example 12Acute toxicological study

This study was conducted in order to observe acute changes in the rabbit nasal cavity after a single dose of vehicle in each nasal cavity. Vehicles tested were glycofurol 75, tetraethylene glycol (Fluka) and propylene glycol (DLS-quality, Mecobenzon, Denmark).

16 rabbits were randomized into 4 groups of 4 rabbits each. 3 groups received a single intranasal application of one of the test vehicles, 50 μ l into each nasal cavity. Each rabbit received only one test compound. One group served as a control receiving the same volume of isotonic sodium chloride saline. 10 minutes after application the rabbits were sacrificed and bled. The right nasal cavity was then opened and evaluated macroscopically. The evaluator was blind as to the dosing scheme. The left nasal cavity was dissected and fixed in neutral buffered formalin for histological evaluation.

Macroscopic and microscopic observations in each rabbit 1-16 are given in table 8 and summarized in table 9 with respect to the four vehicles. Amazingly no macroscopic or microscopic difference was seen between isotonic saline and the 3 vehicles tested.

Table 8
OBSERVATIONS OF RABBIT NASAL CAVITIES
10 MINUTES AFTER INTRANASAL APPLICATION OF VEHICLE

N.A. = No Abnormalities L = Level

5	Macroscopic Observations (Right Cavity)	Microscopic Observations (Left Cavity)
10	1. N.A.	L 1 Congestion L 2 Focal lymphoid aggregates L 3 N.A. L 4 N.A.
15	2. Slightly hyperaemic	L 1 Congestion L 2 Focal haemorrhage; congestion L 3 Focal lymphoid aggregates L 4 N.A.
20	3. N.A.	L 1 Congestion L 2 Congestion L 3 Congestion L 4 Focal submucosal haemorrhage
25	4. Mucous lining nasal cavity more pale than normal	L 1 Congestion L 2 Congestion L 3 Congestion and mucous inspissation L 4 N.A.
30	5. 2 mm haemorrhagic focus in anterior	L 1 Congestion L 2 Congestion, focal haemorrhage and desquamation, edema, small focus of chronic inflammation and focal lymphoid patches L 3 Lymphoid aggregates L 4 Area of chronic inflammation
35		
40	6. 6 x 3 mm area of haemorrhage anterior	L 1 Congestion and a focus of desquamation, minute haemorrh. L 2 Congestion and area of submucosal haemorrhage L 3 N.A. L 4 Slight congestion
45	7. N.A.	L 1 Congestion L 2 Edema, congestion, focal haemor
50		L 3 Congestion, focal lymphoid aggr L 4 Diffuse acute and chronic inflammation. Poly & mono nucl. cell. Blood in lymphatics.

Table 8 (cont.)

OBSERVATIONS OF RABBIT NASAL CAVITIES

10 MINUTES AFTER INTRANASAL APPLICATION OF VEHICLE

N.A. = No Abnormalities L = Level

5	Macroscopic Observations (Right Cavity)	Microscopic Observations (Left Cavity)
10	8. N.A.	L 1 Congestion
		L 2 Congestion with some submucosal haemorrhage, edema
15		L 3 Congestion with some submucosal haemorrhage, edema
	9. N.A.	L 4 Congestion with some submucosal haemorrhage, edema. Mucous inspissation submucosal lymphoid aggregates
20		L 1 Congestion and edema
		L 2 Congestion and edema
25	10. N.A.	L 3 Congestion and lateral wall mono & polymorphonuclear cell infiltrate with lymphoid aggregate
		L 4 Congestion and lateral wall mono & polymorphonuclear cell infiltrate with lymphoid aggregate
30		L 1 Congestion
	11. N.A.	L 2 Congestion with occasional focal lymphoid aggregations
35		L 3 Edema and congestion
		L 4 Focal chronic mononuclear cell infiltrate; dilatation of lymphatics
40	12. N.A.	L 1 Edema and congestion
		L 2 Edema, congestion, focal haemorrhage and submucosal lymphoid aggregates
		L 3 Dilatation of venules
45		L 4 N.A.
		L 1 Congestions; venule dilatation, focal haemorrhage
50		L 2 Congestion; edema, focal haemorrhage
		L 3 Congestion; submucosal lymphoid aggregates
		L 4 Congestion; submucosal lymphoid aggregates

Table 8 (cont.)

OBSERVATIONS OF RABBIT NASAL CAVITIES

10 MINUTES AFTER INTRANASAL APPLICATION OF VEHICLE

5 N.A. = No Abnormalities L = Level

	Macroscopic Observations (Right Cavity)	Microscopic Observations (Left Cavity)
10	13. N.A.	L 1 Congestion
		L 2 Congestion, edema, submucosal lymphoid aggregates
15		L 3 Congestion, edema, submucosal lymphoid aggregates
		L 4 Congestion and pronounced lymphoid aggregates
20	14. 3 mm haemorrhagic focus towards anterior	L 1 N.A.
		L 2 Congestion, edema, submucosal lymphoid aggregates
25		L 3 Congestion, edema, submucosal lymphoid aggregates
		L 4 Congestion, edema, submucosal lymphoid aggregates
30	15. A small abrasion, pinpoint in the anterior area	L 1 Congestion, edema, focal haemorrhage
		L 2 Congestion, focal haemorrhage, edema, submucosal lymphoid aggregates
35		L 3 Congestion, edema, mononuclear cell infiltrate near basal section
		L 4 Congestion
40	16. 4 x 3 mm area of haemorrhage towards anterior	L 1 Congestion; edema
		L 2 Congestion, edema, focal haemorrhage and desquamation
45		L 3 Congestion, edema, submucosal lymphoid aggregates, especially near base
		L 4 Edema, submucosal lymphoid aggregates

Table 9

SUMMARY OF RESULTS OF RABBIT NASAL CAVITIES 10 MINUTES
AFTER INTRANASAL APPLICATION OF 4 EGf, GF, PG AND 0.9% NaCl
(ARRANGED BY VEHICLE)

5

N.A. = No Abnormalities

	Vehicle	Animal No.	Macroscopic Results (Right side)	Microscopic Results (Left side)
10	0.9% NaCl	3	N.A.	Focal haemorrhage
		6	6x3 mm haemorrhagic focus, ant. section	Focal haemorrhage
15		12	N.A.	Focal haemorrhage
		13	N.A.	N.A.
20	Glyco-furol (GF)	2	N.A.	Focal haemorrhage
		8	N.A.	Focal haemorrhage
		9	N.A.	N.A.
25		16	4x3 mm haemorrhagic focus, ant. section	Focal haemorrhage
30	Propylene glycol (PG)	4	N.A.	N.A.
		5	2 mm haemorrhagic focus, ant. section	Focal haemorrhage
		11	N.A.	Focal haemorrhage
35		14	2 mm haemorrhagic focus. ant. section	N.A.
40	Tetra-ethylene glycol (4 EGf)	1	N.A.	N.A.
		7	N.A.	Focal haemorrhage
		10	N.A.	N.A.
45		15	Pinpoint abrasion, anterior	Focal haemorrhage

Surprisingly it has been found that the intranasal absorption of e.g. benzodiazepines, such as clonazepam and diazepam, in the vehicles according to the invention is very similar to an intravenous injection (i.v.). From Figure 1 it appears that the peak clonazepam plasma concentration (t_{max}) is reached within less than 2 - 3 minutes and that the plasma concentration after 2½ minutes ($C_{pl} (2\frac{1}{2} \text{ min})$) is about 100% of that obtained by i.v. administration. The choice of the quality of the vehicles, according to the invention, had surprisingly an influence on the rate of absorption. The plasma concentration of clonazepam at 2½ minute is e.g. about 40% higher for 4EGf (quality from Fluka-Chemie AG) than for 4EGm (quality from MERCK-Schuchardt) or PEG 200 (from MERCK-Schuchardt), and t_{max} is about ≤2½, 10 and 15 minutes, respectively (Figure 5).

15 The pharmacodynamic response was studied in rabbits. The time to response after intranasal application of 0.25 mg clonazepam in 100 µl of vehicle was measured. The mean times are given in Figure 2 and the experimental details are stated in Example 3. As shown in Figure 2, a pharmacodynamic response after 20 intranasal administration of clonazepam in the vehicles according to the invention may be obtained in less than 2 minutes after the application.

The compositions of the invention are stable. This has e.g. been demonstrated by measuring benzodiazepine concentration of 25 the compositions according to the invention after a month at 60°C and at 25°C, respectively (see example 11). A stable solution (recovery about ≥90%) can be obtained using PG after addition of acetic acid (adjusted to pH 3.8 - 4). The same amount of acetic acid was not able to render the 4EGf solution 30 stable, and the amount of acetic acid needed for the adjusting pH to about 4 was too high. Amazingly adjusting pH using nitric acid resulted in stable solutions of clonazepam in e.g. 4EGf optionally comprising 5% GF (recovery about ≥90%).

Surprisingly, it has also been found that using the vehicles according to the invention together with insulin or glucagon a pronounced biological response is obtained within 15-30 minutes after intranasal application to rabbits (Figure 3 and 4, and 5 examples 4 and 5).

Example 13

Bioavailability

The bioavailability of estrogen (E2) and estrone (E1), after intranasal (i.n.) application to rabbits (n=3) of a single dose of 50 µg E2, was studied in pilot. Two formulations containing 30% and 100% glycofurol (GF), respectively, were tested i.n. relative to an i.v.-injection of the same dose.

Materials

17-β-estradiol (estrogen=E2) was obtained from Novo Nordisk (Copenhagen, Denmark), propylenglycolum ad infundibilia (PG) from Mecobenzon (Copenhagen, Denmark) and glycofurolum 75 (GF) from Hoffman La-Roche (Basel, Switzerland). All other reagents were of reagent or analytical grade.

Drug preparation

The formulations for i.v.-injection and for intranasal application were prepared just before the administration. Formulation 1 for i.v. administration was prepared by dissolving 2.729 mg E2 in 25.0 ml PG and then adding 25.0 ml isotonic saline. Formulation 2 for i.n. application was prepared by dissolving 4.938 mg E2 in 10.0 ml GF. Formulation 3, also for i.n. application, was prepared by dissolving 4.996 mg E2 in 3.00 ml GF and then adding isotonic saline to a total volume of 10.0 ml.

Dosing and study design

Rabbits (n=3) having i.v. administration received 1.0 ml of formulation 1 i.v. (equivalent to 50 μ g E2) as an ear-vein infusion during 30 seconds. Rabbits (n=3) having i.n. administration received formulation 2 or 3 i.n. with an Eppendorph pipette. Each rabbit received 50 μ l into each nostril, equivalent to 50 μ g E2. The intranasal application occupied about 5 seconds. During and about 1 minute after the intranasal applications, the rabbits were held in a supine position.

10 Blood samples were collected 0, 5, 10, 20, 30, and 60 minutes after the administration. Plasma was isolated and stored at $\pm 20^{\circ}\text{C}$ until analysis.

The plasma concentrations of unconjugated E2 and E1 were measured by radioimmunoassay as described by Emmet et al., 15 1972. After extraction with ether, separation of E1 and E2 was performed on columns of Sephadex LH20. Radioimmunoassay was performed after evaporation of the solvents. The detection limits for E1 and E2 were 40 pmol/l. The intra- and interassay variance for E1 were 7.0% and 9.6%, and for E2 7.4% and 10.5%, 20 respectively.

Dose/weight correction for individual plasma concentrations was calculated relative to the mean of i.v. dose/weight (22.3 μ g/kg). For formulation 1 i.v. the area (AUC) under the E2 plasma concentration-time curves, from 0 min to infinity, a one 25 compartement with 30 seconds infusion was fitted to the data. All other AUC's were calculated by means of the trapezoidal rule.

The plasma concentrations E2 and E1 after administration of E2 appear from Table 10 and 11 and figures 11 and 12, respectively. Figure 11 shows the mean \pm S.D. estrogen (E2) plasma concentrations after administration of about 50 μ g estrogen to 5 rabbits (n=3) as an i.v.-injection (formulation 1) or intranasal administration (2 i.n. and 3 i.n. formulated with glycofurol (GF) 100% and 30%, respectively).

___x___ form. 1 i.v., ___●___ form. 2 i.n., and
...o... form. 3 i.n.,

10 and figure 12 shows the mean \pm S.D. estrone (E1) plasma concentrations after administration of about 50 μ g estrogen to rabbits (n=3) as an i.v.-injection (formulation 1) or intranasal administration (2 i.n. and 3 i.n. formulated with glycofurol (GF) 100% and 30%, respectively).

15 ___x___ form. 1 i.v., ___●___ form. 2 i.n., and
...o... form. 3 i.n.

As seen from Figs. 11 and 12 and Table 12, intranasal application was very similar to i.v administration. The bioavailability appears from Table 12. The bioavailability for E2 was 87 and 20 80%, and for E2+E1 104 and 95% for formulation 2 i.n. and 3 i.n., respectively. C_{5min} for E2 was 109% and 95%, and for E2+E1 118% and 105%. T_{max} was shorter than 5 min.

Table 10

Individual estrogen (E2) plasma concentrations after administration of about 50 µg estrogen to rabbits (n=3) as an i.v.-injection (formulation 1) or intranasal administration (2 i.n. and 3 i.n. formulated with glycofurol (GF) 100% and 30%, respectively).

5 n.d.=lower than 0.04 nmol/ml.

Formulation	Rabbit no.	E2 plasma concentration (nmol/ml) after min.						Dose/weight pg/kg
		0	5	10	20	30	60	
1. i.v.	4	n.d.	49.0	37.0	19.0	15.0	4.90	22.2
	5	0.12	65.0	45.0	29.0	18.0	7.60	21.8
	6	0.12	38.0	30.0	25.0	21.0	11.0	22.9
2. i.n. (100% GF)	1	0.19	75.0	26.0	16.0	11.0	3.70	20.1
	2	0.09	50.0	32.0	18.0	11.0	5.20	21.3
	3	n.d.	44.0	24.0	14.0	8.8	3.30	19.3
3. i.n. (30% GF)	7	0.10	41.0	31.0	15.0	8.7	3.90	17.5
	8	0.09	64.0	32.0	18.0	10.0	3.40	19.4
	9	0.08	31.0	17.0	8.2	5.9	1.90	18.5

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Table 11

Individual estrone (E1) plasma concentrations after administration of about 50 µg estrogen to rabbits (n=3) as an i.v.-injection (formulation 1) or intranasal administration (2 i.n. and 3 i.n. formulated with glycofurol (GF) 100% and 30%, respectively).

5 n.d.=lower than 0.04 nmol/ml.

Formulation	Rabbit no.	E1 plasma concentration (nmol/ml) after min.						Dose/weight pg/kg
		0	5	10	20	30	60	
1. i.v.	4	n.d.	4.30	4.30	3.10	2.70	1.60	22.2
	5	n.d.	4.00	5.10	4.60	3.30	1.50	21.8
	6	n.d.	2.20	2.60	2.80	2.30	1.50	22.9
2. i.n. (100% GF)	1	n.d.	11.00	9.90	7.10	4.80	3.00	20.1
	2	n.d.	6.20	7.40	6.00	3.40	3.00	21.3
	3	n.d.	8.50	9.00	5.90	4.30	1.60	19.3
3. i.n. (30% GF)	7	n.d.	8.10	8.90	5.80	3.90	2.30	17.5
	8	n.d.	5.30	6.10	5.00	3.90	2.40	19.4
	9	n.d.	8.80	7.50	3.80	3.70	1.90	18.5

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Table 12

Pharmacokinetic data (dose corrected relative to mean i.v. dose) for estrogen (E2) and estrone (E1) after single administration of about 50 µg estrogen (n=3) as an i.v.-injection (1 i.v.) or intranasal administration (2 i.n. and 3 i.n.).

AUC (nmol ml⁻¹ min) is the area under the plasma concentration - time curve from 0 min to infinity.

C_{max} (nmol/ml) is the maximal plasma concentration.

t_{max} (min) is the time to maximal plasma concentration.

Parameter	Observed mean (dose corrected)			% Relative to 1 i.v.-mean ± SD	
	1 i.v.	2 i.n.	3 i.n.	2 i.n.	3 i.n.
AUC	E ₂	1302	1138	1038	80±30
	E ₁	241	471	432	195±18
	E ₂ +E ₁	1543	1609	1470	104±13
C _{max}	E ₂	57	62	54	109±29
	E ₁	4	10	10	150±22
	E ₂ +E ₁	61	72	64	118±28
t _{max}	E ₂	5	5	5	100± 0
	E ₁	12	8	8	67±38

COMMENTS

The solubility for E2 in GF is found to be about 230 mg/ml. Thus, a clinical dose of 50 μ g is soluble in 0.25 μ l. Hence, the application of a clinical dose in an extremely small μ l-
5 volumen is rendered possible using GF. As the solubility decreases exponentially in combination with water, the dose volume using GF as a vehicle should e.g. be about 5 μ l, to avoid unwanted precipitation of E2 in the nasal mucus.

A small dose volume is desirable in order to reduce or elimina-
10 te a local irritating effect. Alternatively a non irritating co-solvent, e.g. vegetabile oil, may be added. In this way a desired dose volume or delivery rate may also be obtained. To reduce plasma peak concentration, a limited precipitation of E2 in the mucus may also be desirable.

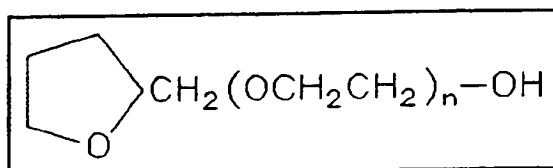
15 A anhydrous GF-formulation may be useful in acute hot flushing as well as in chronic dosing.

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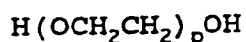
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C l a i m s

1. A pharmaceutical composition for application of biologically active substance(s) to a mucosal membrane of a mammal comprising one or more substance(s) selected from the group consisting of n-glycofurols represented by the formula I:



wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:



wherein p is 1 to 14.

2. A pharmaceutical composition according to claim 1, wherein the n-ethylene glycol(s) comprise(s) n-ethylene glycols where p is 1-8.

3. A pharmaceutical composition according to any of claims 1 or 2, wherein n is the n-glycofurols is 1 or 2.

4. A composition according to any one of claims 1-3 wherein the biologically active substance(s) is (are) selected from biologically active peptides which are digested in the gastrointestinal tract.

5. A composition according to claim 4 comprising a biologically active substance selected from the group consisting of coagulation factors such as Factor VII, Factor VIII, Factor IX and derivatives and analogues thereof; agents controlling bone metabolism such as calcitonin and derivatives and analogues thereof; hormones secreted by hypothalamus such as LHRH or analogues, e.g. nafarelin, buserelin or Zolidex, enkephalins such as DADLE, metkephamid or leucine enkephalin; hormones

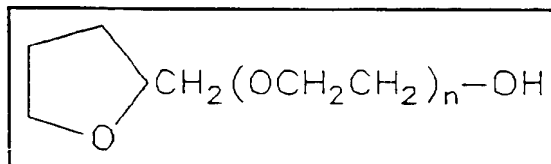
secreted by pancreas such as insulin or glucagon and derivatives and analogues thereof; hormones secreted by the pituitary gland such as growth hormone, vasopressin or oxytocin and derivatives and analogues thereof, e.g. DDAVP or lypressin; 5 sex-hormones such as FSH, LH or LTH and derivatives and analogues thereof.

6. A composition according to any of claims 1-3 wherein the biologically active substance(s) is(are) selected from anti-epileptics, spasmolytics and tranquillisers selected from the 10 group of benzodiazepines such as clonazepam, diazepam, flunitrazepam, triazolam, lorazepam, nitrazepam or mixtures thereof, in an amount of from 0.001% to 20%.

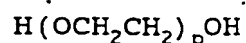
7. A composition according to any of claims 4-6 further comprising nitric acid and/or nitrate in a concentration 15 ranging from 0.0001 to 5%.

8. A composition according to claim 4 or 5, comprising pancreatic hormones such as insulin and derivatives and analogues thereof, and glucagon and derivatives and analogues thereof.

9. A method for treatment of a mammal with a biologically 20 active substance wherein the biologically active substance is applied to a mucosal membrane of the mammal to be treated together with one or more substance(s) selected from the group consisting of n-glycofurols represented by the formula I:

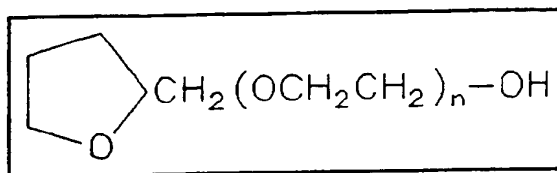


wherein n is 1 to 8, and n-ethylene glycols represented by the 25 formula II:

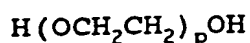


wherein p is 1 to 14.

10. Use of a pharmaceutical composition comprising biologically active substance(s) and one or more substance(s) selected from group consisting of n-glycofurols represented by the formula I:

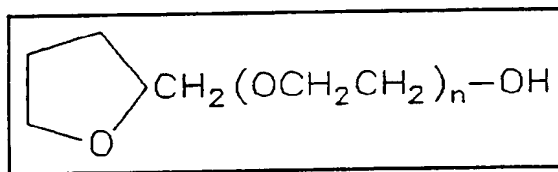


5 wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:

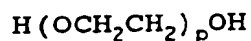


wherein p is 1 to 14 for application to a mucosal membrane.

11. A method for producing a pharmaceutical composition for
10 application of a biologically active substance to a mucosal membrane of a mammal comprising one or more substance(s) selected from group consisting of n-glycofurols represented by the formula I:



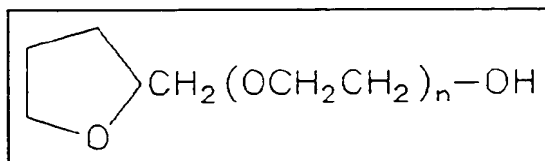
15 wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:



wherein p is 1 to 14, wherein the biologically active substance is dissolved in a prepared vehicle comprising a part of or all the remaining constituents of the pharmaceutical preparation.

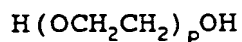
20 12. A method according to claim 11 wherein the biologically active substance is dissolved using ultrasound and/or at an elevated temperature.

13. Use of one or more substance(s) selected from the group consisting of n-glycofurols represented by the formule I:



wherein n is 1 to 8, and n-ethylene glycols represented by the formula II:

5



wherein p is 1 to 14 for the preparation of a pharmaceutical preparation for application of a biologically active substance to a mucosal membrane of a mammal.

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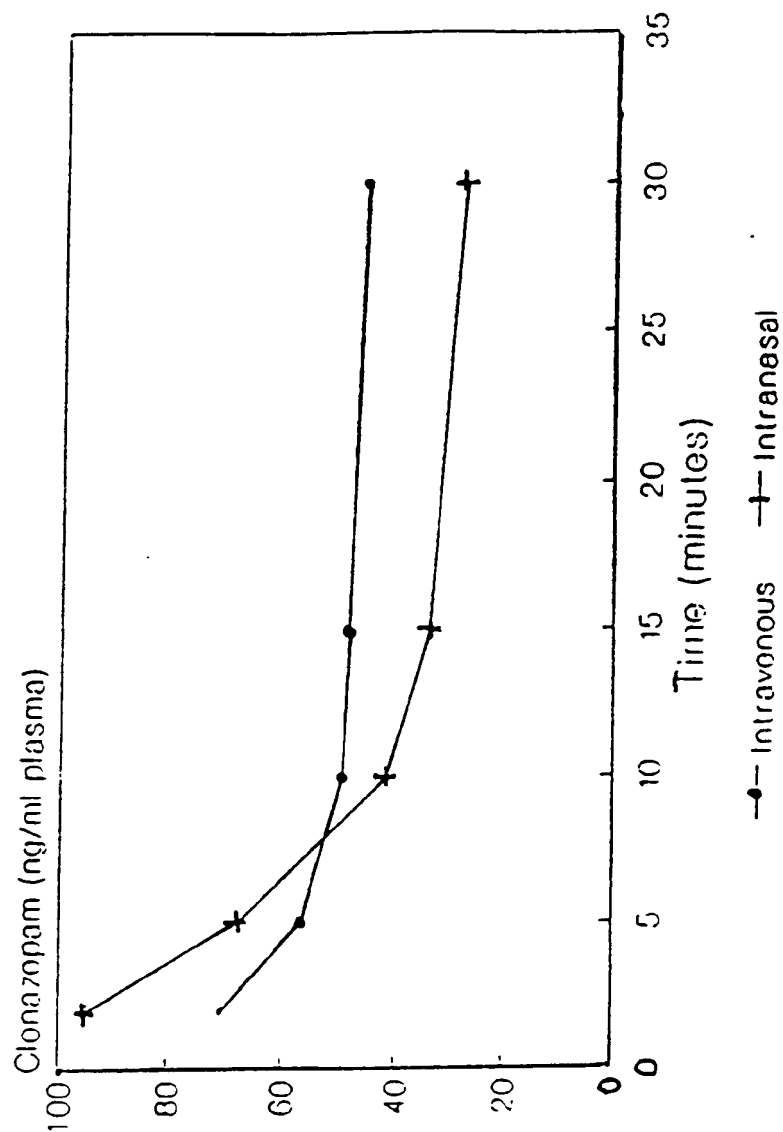


FIG. 1

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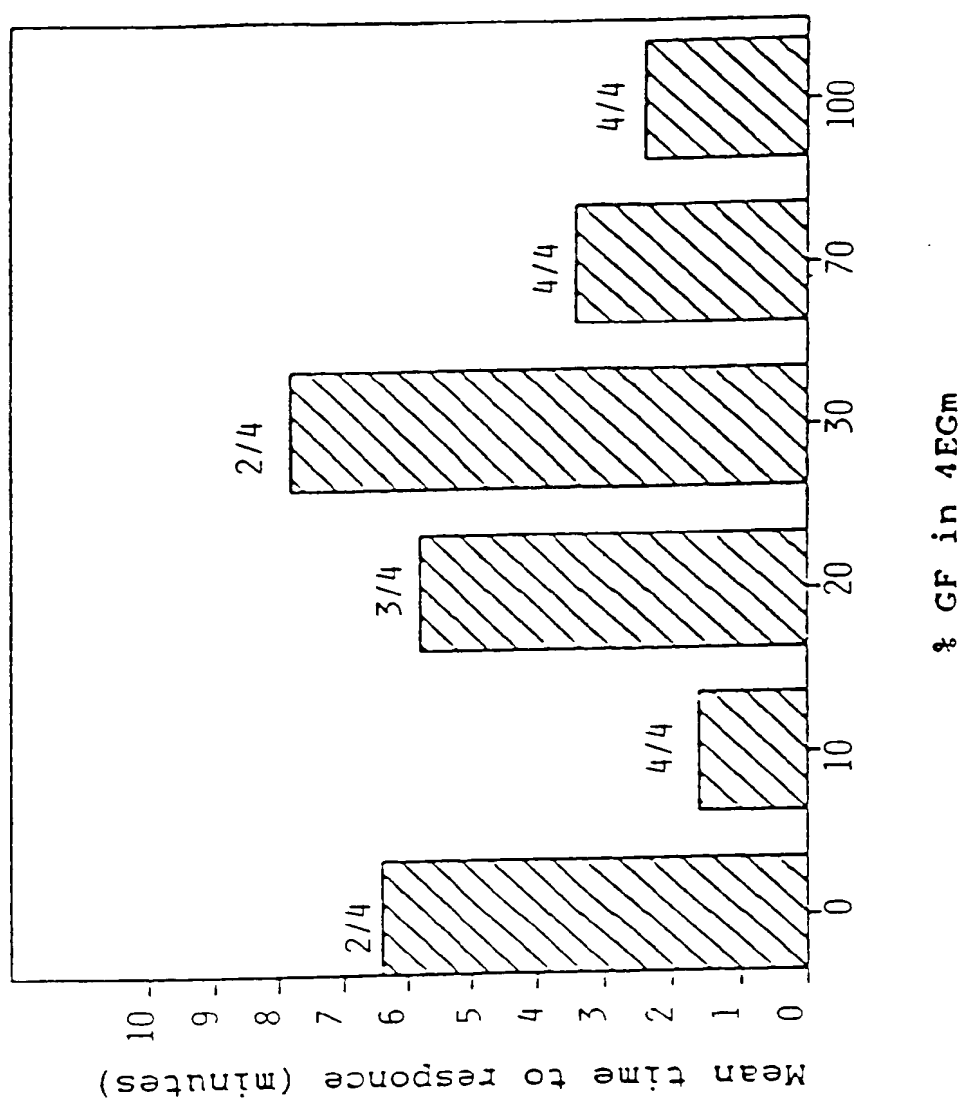


FIG. 2

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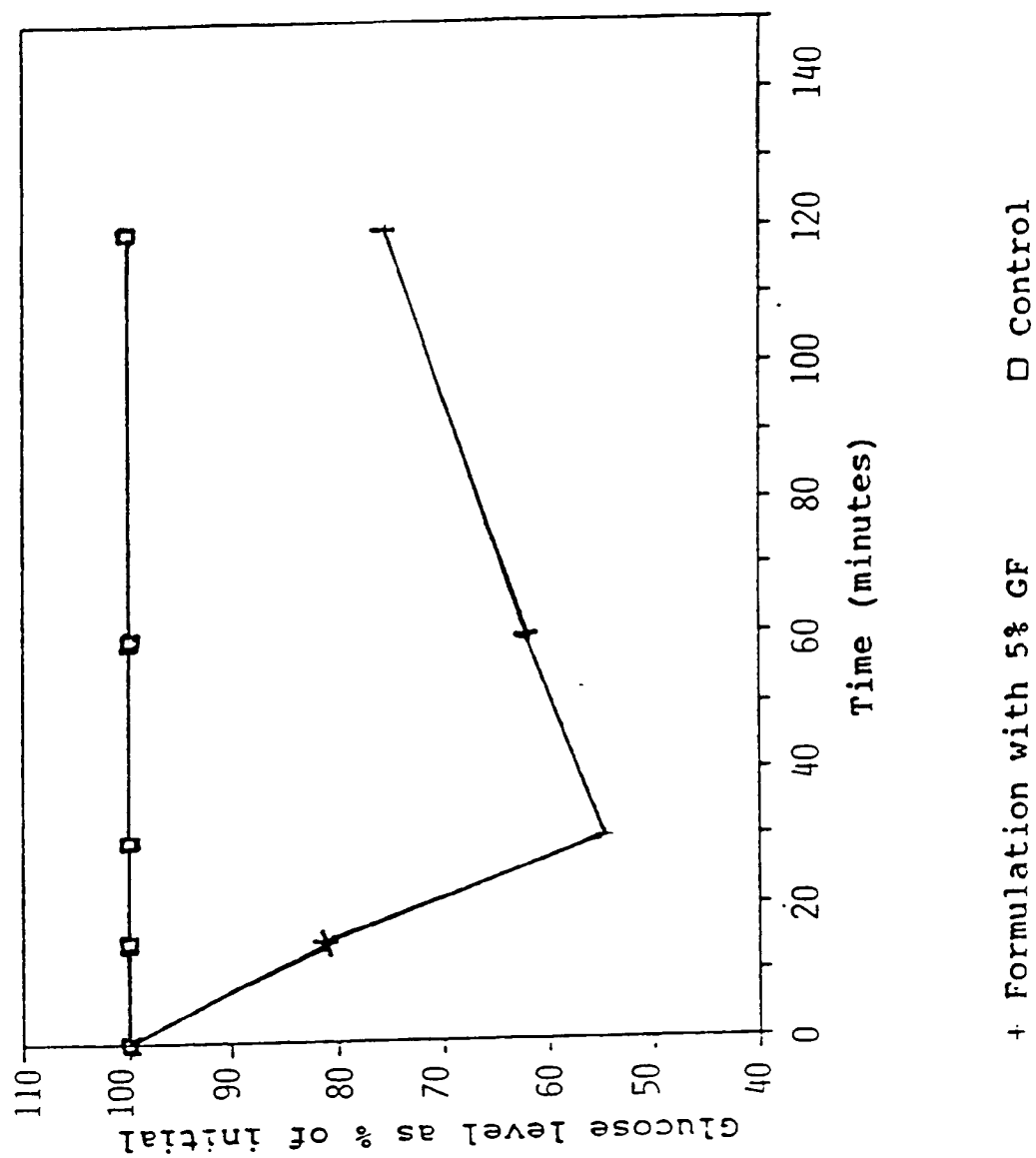


FIG. 3

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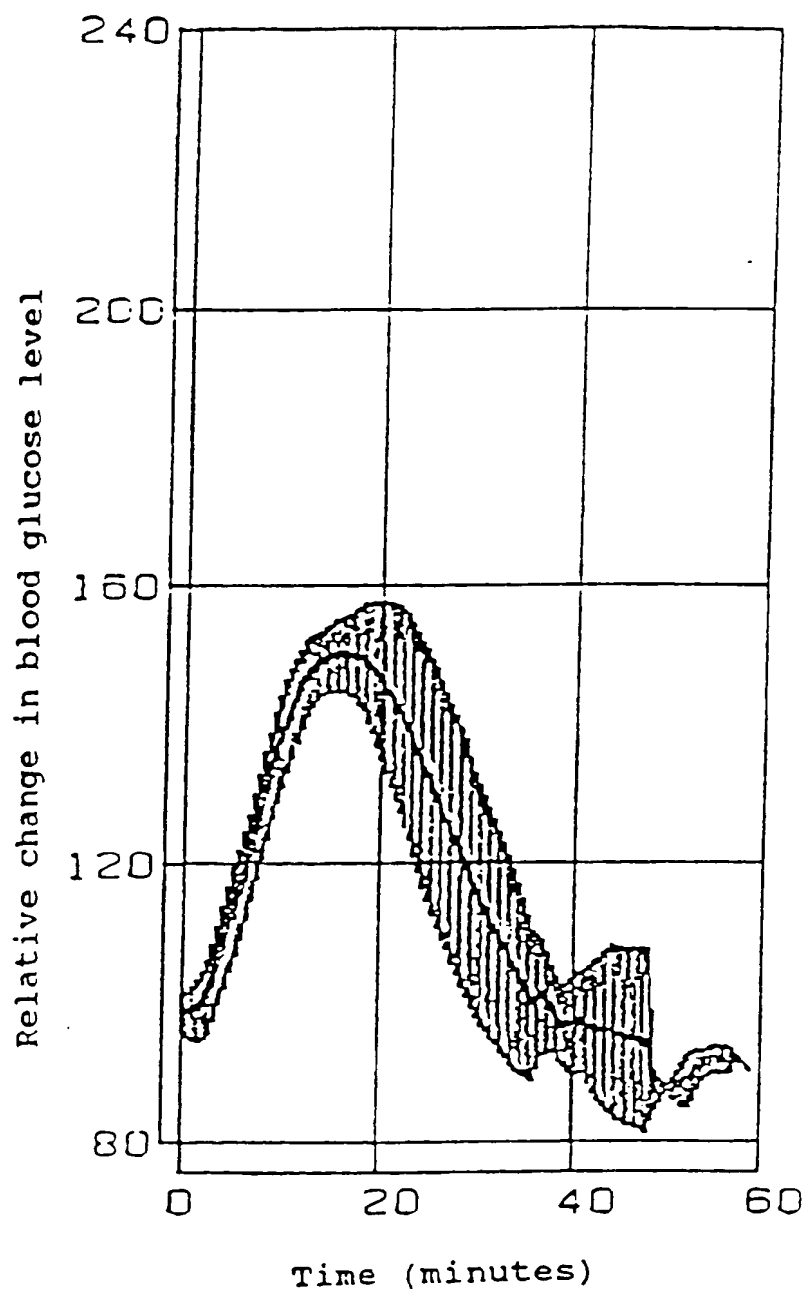


FIG. 4

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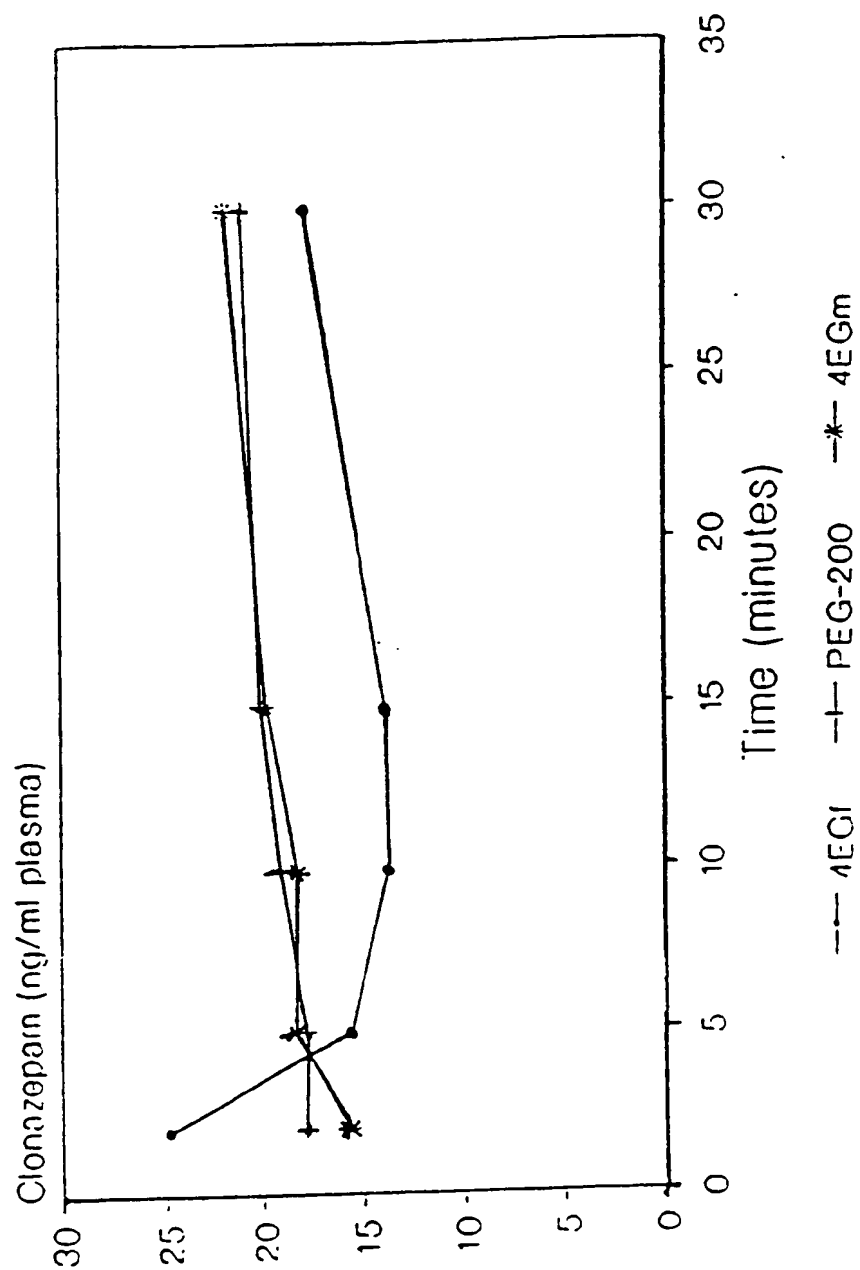


FIG. 5

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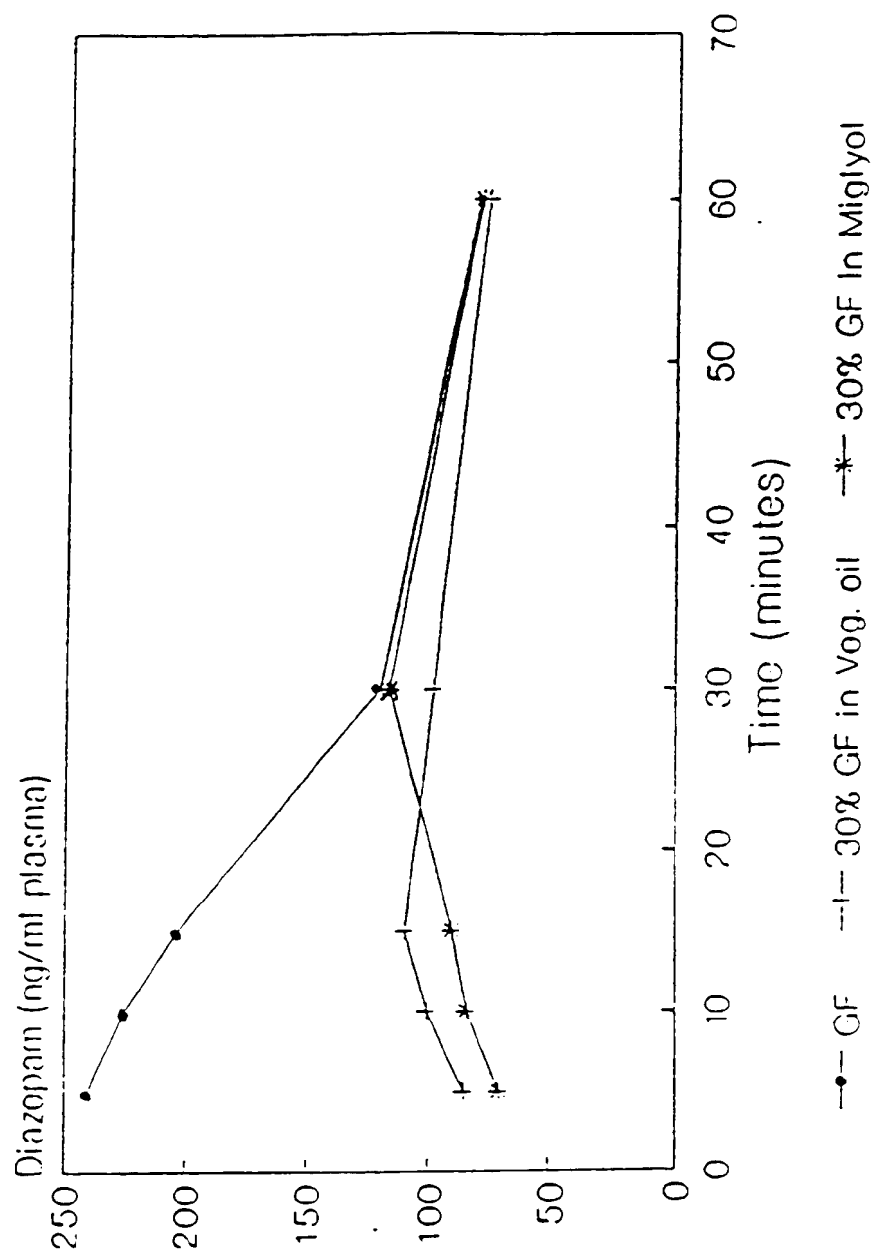


FIG. 6

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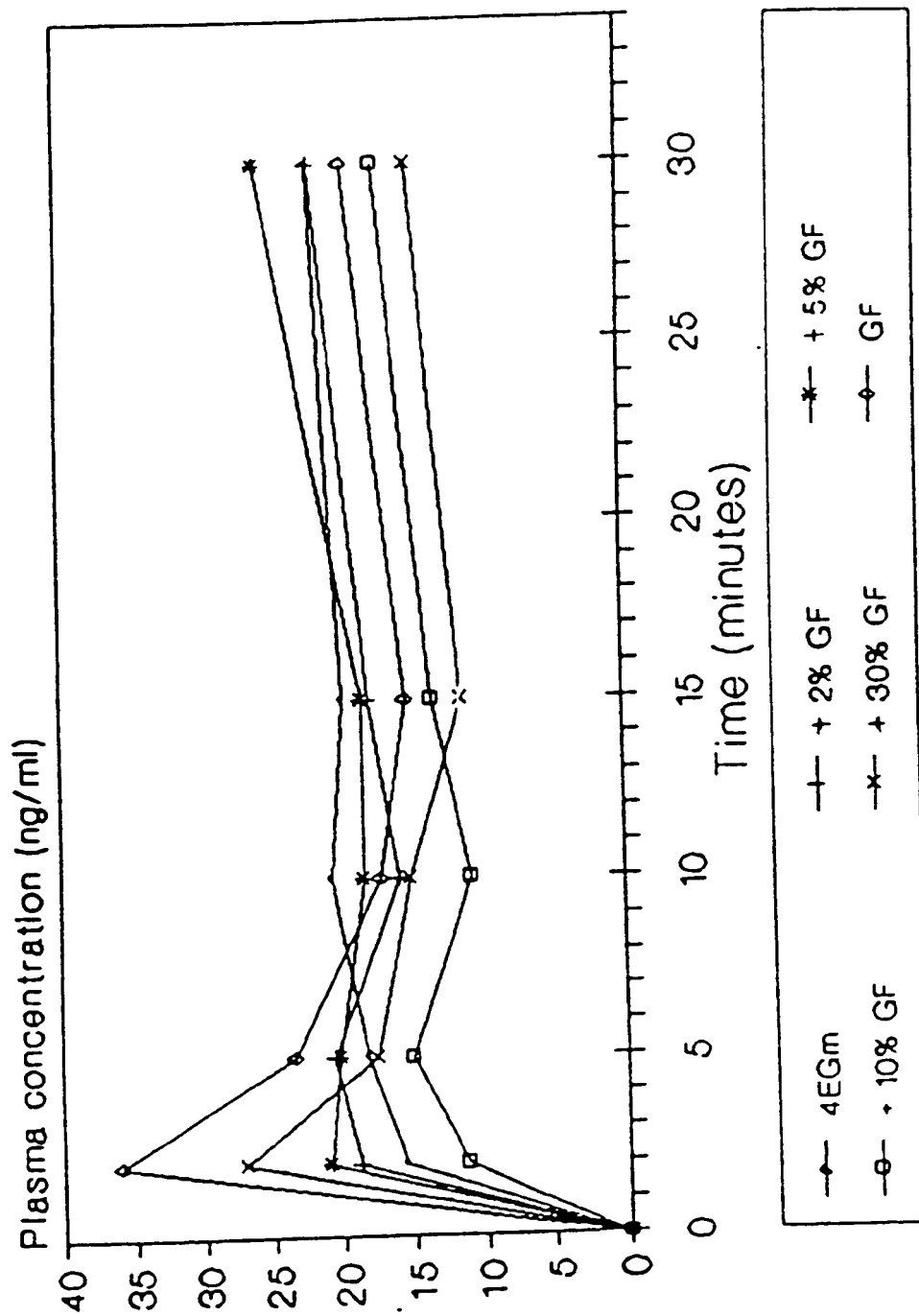


FIG. 7

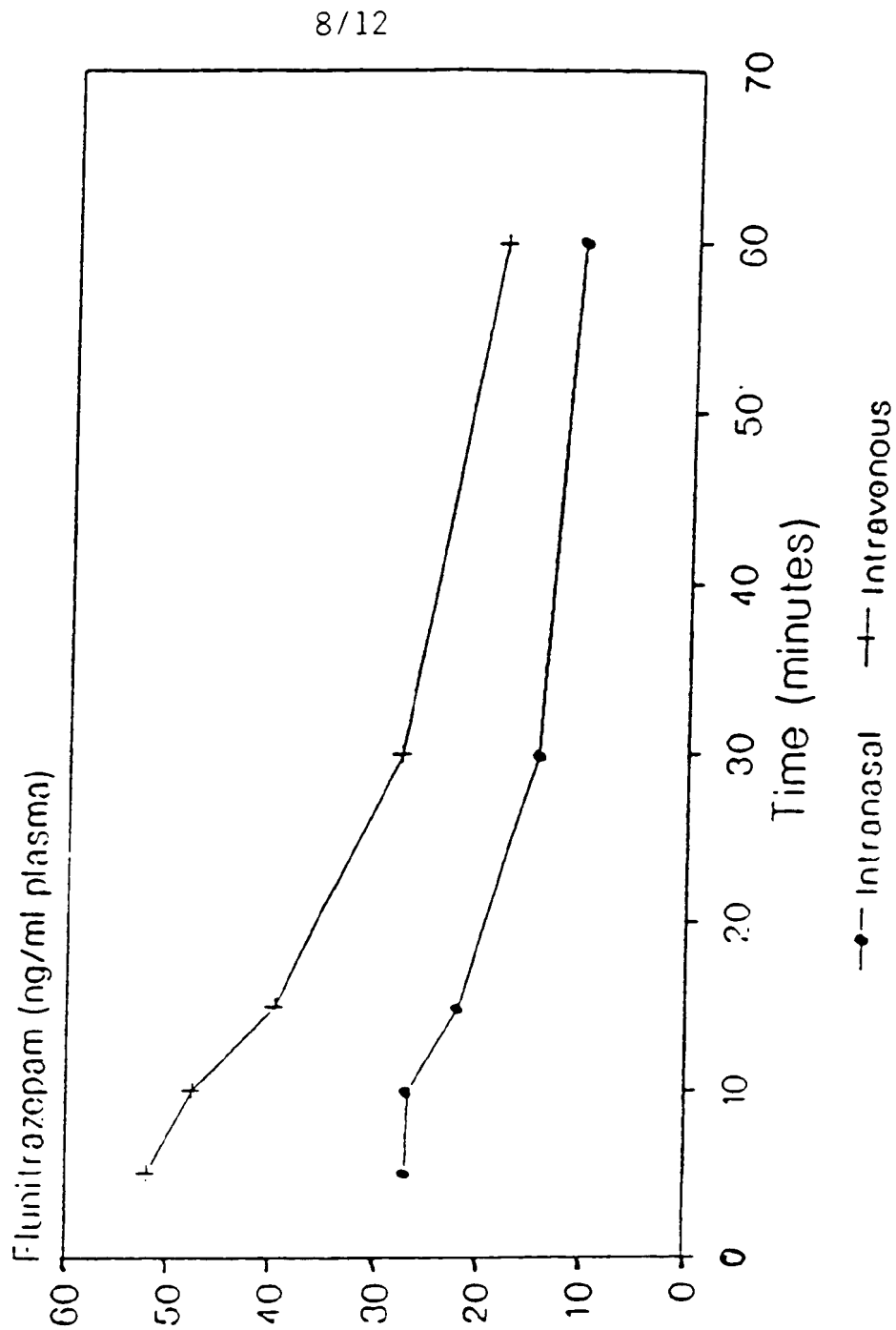


FIG. 8

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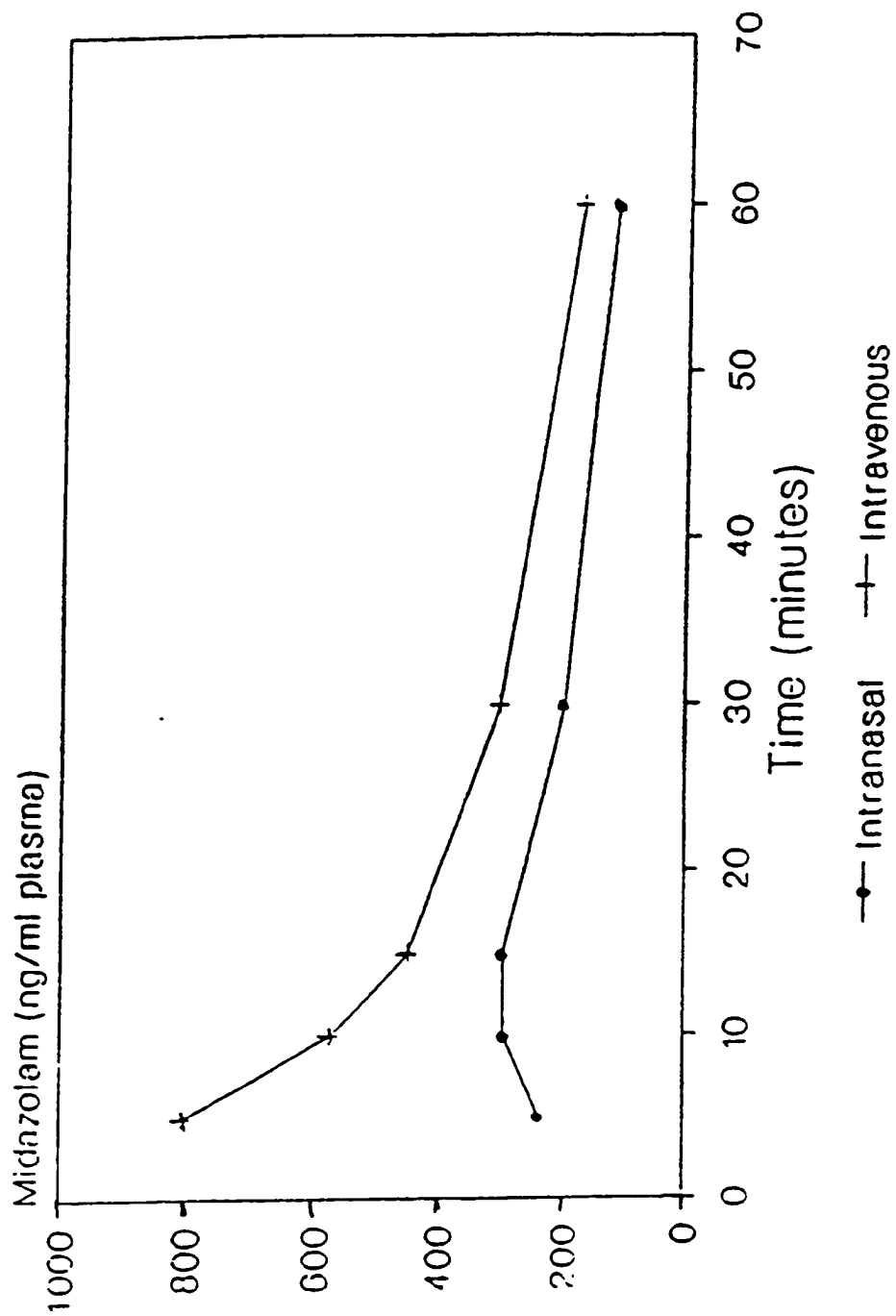


FIG. 9

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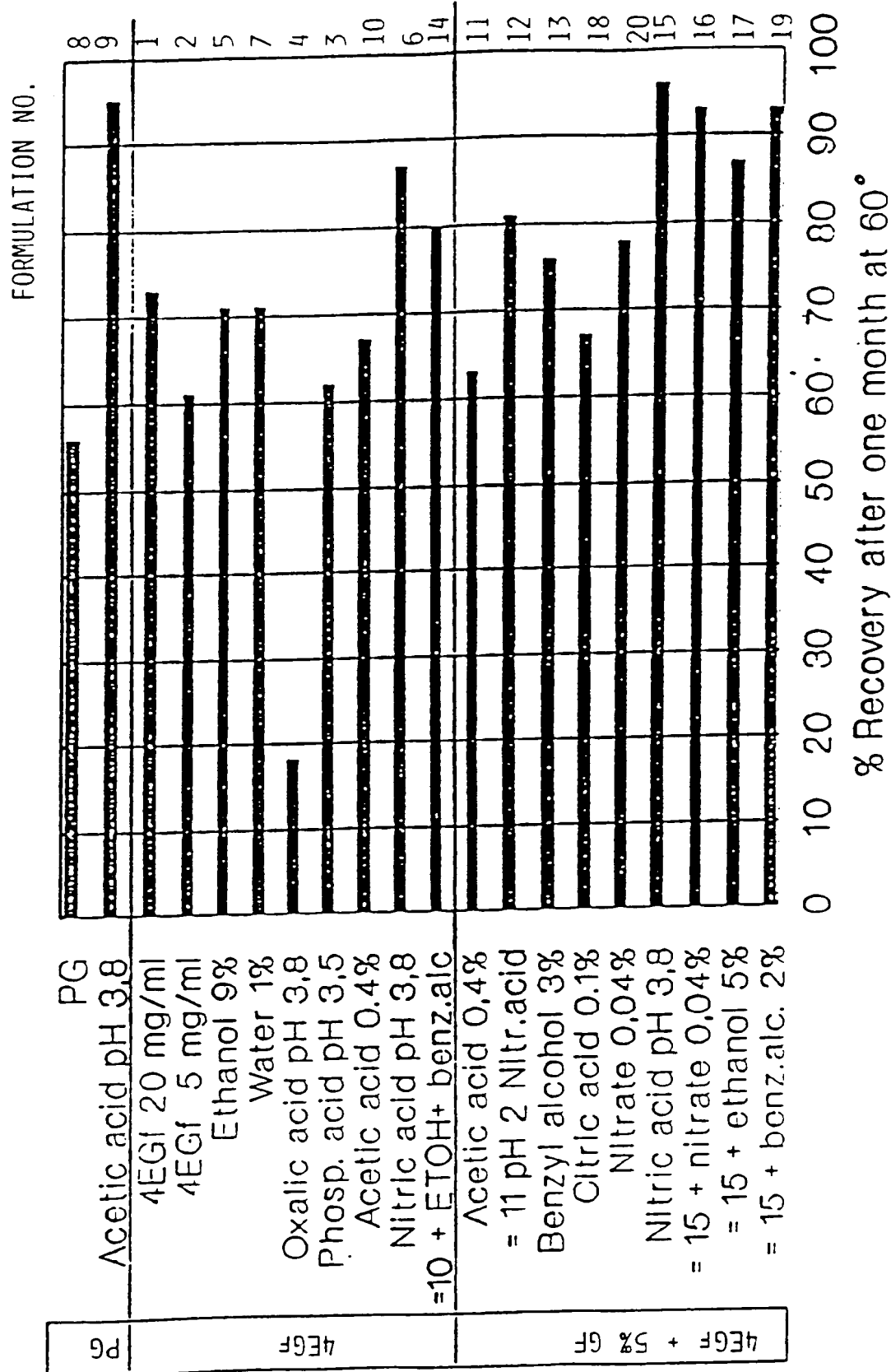


FIG. 10

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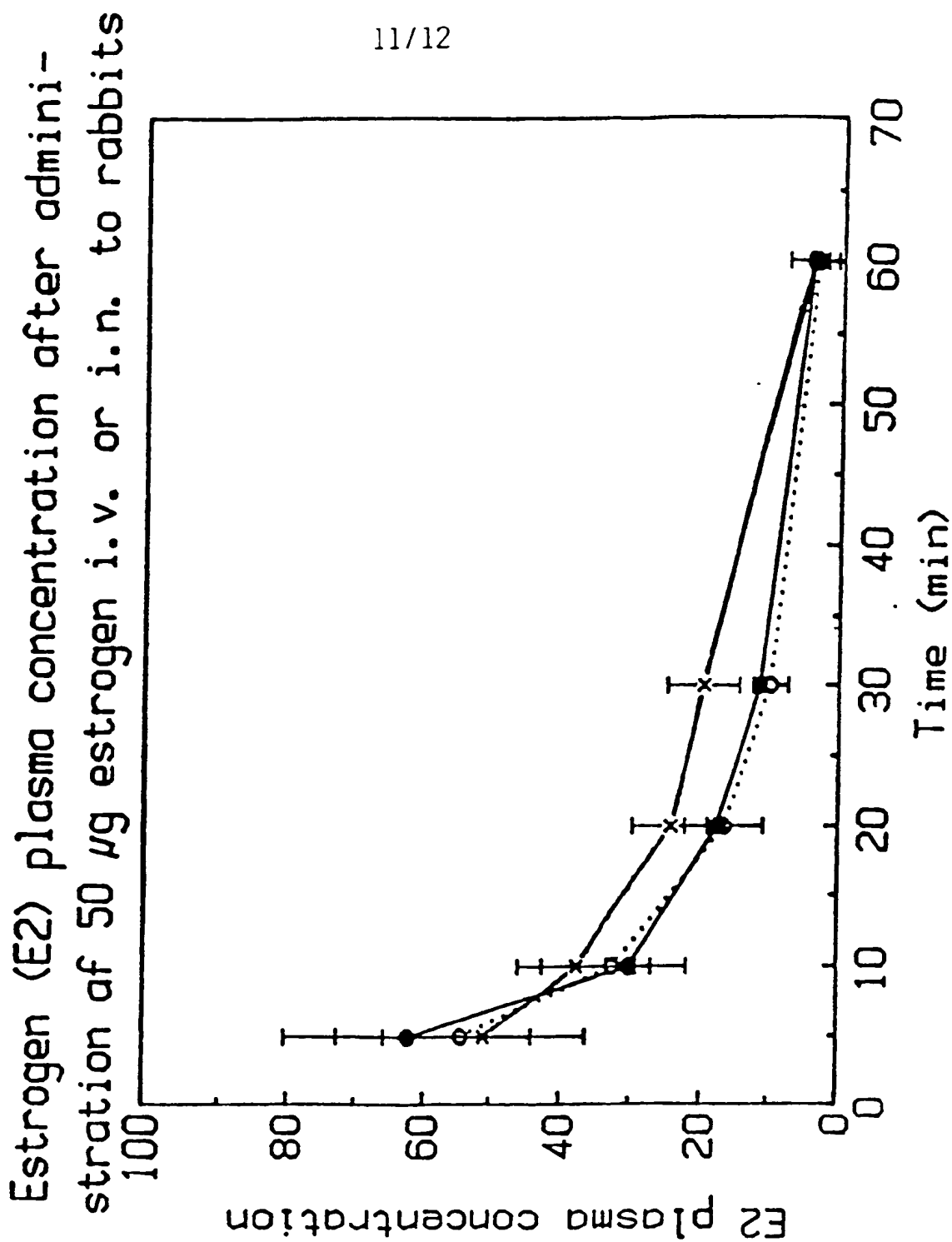


FIG. 11

Estrone (E1) plasma concentration after administration of 50 μ g estrogen i.v. or i.n. to rabbits

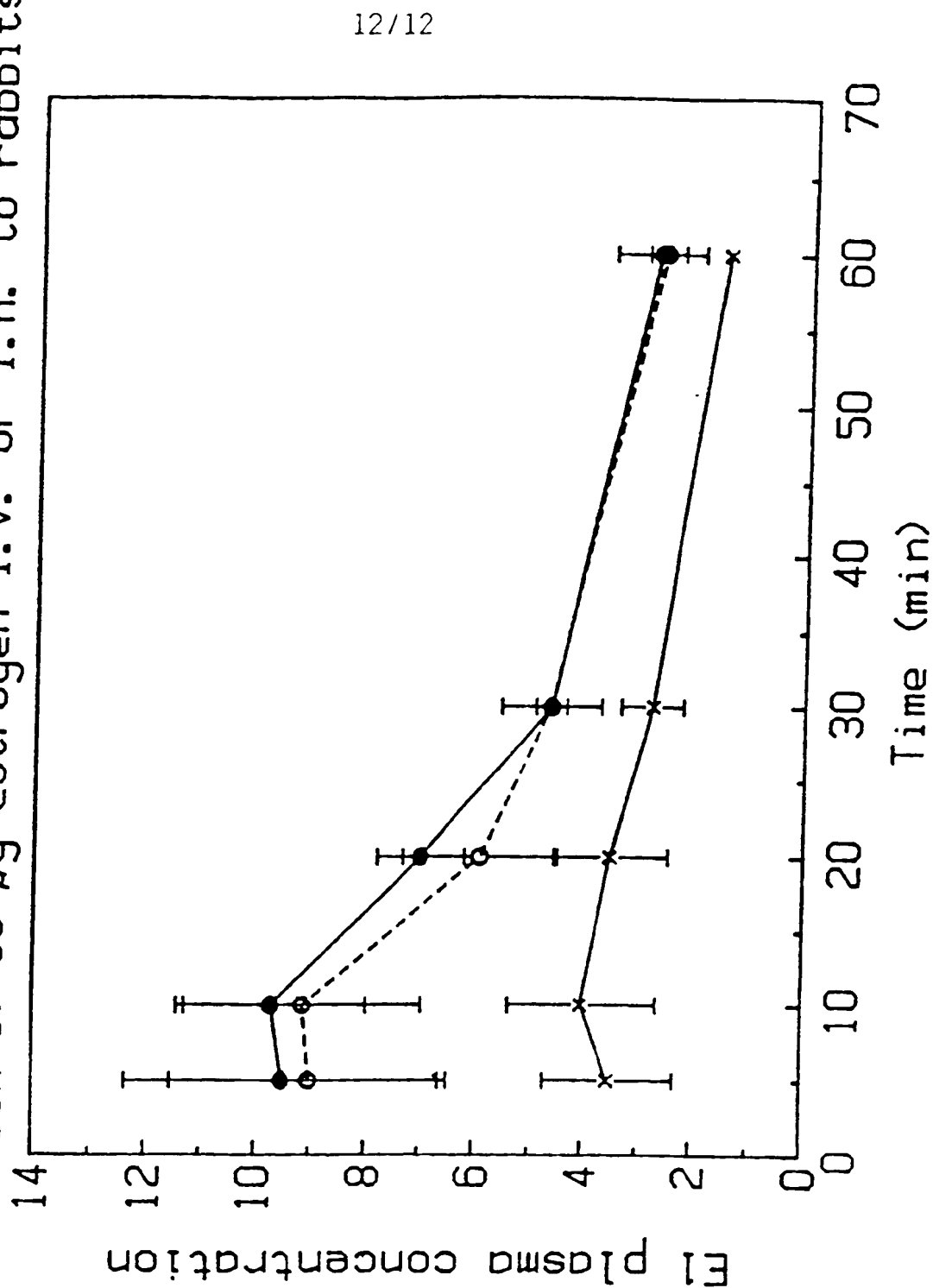
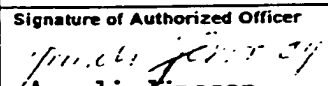
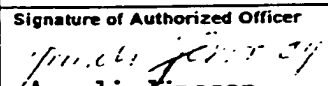
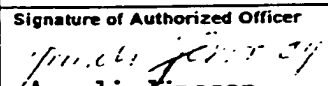


FIG. 12

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK 91/00119

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC5: A 61 K 47/10, 9/06, 9/72											
II. FIELDS SEARCHED <div style="text-align: right; margin-right: 100px;">Minimum Documentation Searched⁷</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 20%; padding: 5px;">Classification System</td> <td style="padding: 5px;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">IPC5</td> <td style="padding: 5px;">A 61 K</td> </tr> </table> <div style="text-align: center; margin-top: 5px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched⁸</div> <p style="margin-top: 10px;">SE,DK,FI,NO classes as above</p>			Classification System	Classification Symbols	IPC5	A 61 K					
Classification System	Classification Symbols										
IPC5	A 61 K										
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; padding: 5px;">Category *</th> <th style="width: 60%; padding: 5px;">Citation of Document,¹¹ with indication, where appropriate, of the relevant passages¹²</th> <th style="width: 30%; padding: 5px;">Relevant to Claim No.¹³</th> </tr> <tr> <td style="padding: 5px; vertical-align: top;">X</td> <td style="padding: 5px; vertical-align: top;">EP, A2, 0095897 (BEECHAM GROUP PLC) 7 December 1983, see spec. page 2, line 1-24, page 5, line 1-13, example 4, claims --</td> <td style="padding: 5px; vertical-align: top;">1-8,10-13</td> </tr> <tr> <td style="padding: 5px; vertical-align: top;">P,A</td> <td style="padding: 5px; vertical-align: top;">EP, A2, 0430149 (HEINRICH MACK NACHF.) 5 June 1991, see the whole document -- -----</td> <td style="padding: 5px; vertical-align: top;">1-8,10-13</td> </tr> </table>			Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	EP, A2, 0095897 (BEECHAM GROUP PLC) 7 December 1983, see spec. page 2, line 1-24, page 5, line 1-13, example 4, claims --	1-8,10-13	P,A	EP, A2, 0430149 (HEINRICH MACK NACHF.) 5 June 1991, see the whole document -- -----	1-8,10-13
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³									
X	EP, A2, 0095897 (BEECHAM GROUP PLC) 7 December 1983, see spec. page 2, line 1-24, page 5, line 1-13, example 4, claims --	1-8,10-13									
P,A	EP, A2, 0430149 (HEINRICH MACK NACHF.) 5 June 1991, see the whole document -- -----	1-8,10-13									
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"Z" document member of the same patent family</p> </div> </div>											
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search 22nd August 1991 </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report 1991 -08- 30 </td> </tr> <tr> <td style="width: 50%; padding: 5px;"> International Searching Authority <div style="text-align: center;">SWEDISH PATENT OFFICE</div> </td> <td style="width: 50%; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">  Anneli Jonsson </div> </td> </tr> </table>			Date of the Actual Completion of the International Search 22nd August 1991	Date of Mailing of this International Search Report 1991 -08- 30	International Searching Authority <div style="text-align: center;">SWEDISH PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">  Anneli Jonsson </div>					
Date of the Actual Completion of the International Search 22nd August 1991	Date of Mailing of this International Search Report 1991 -08- 30										
International Searching Authority <div style="text-align: center;">SWEDISH PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">  Anneli Jonsson </div>										

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers....9....., because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1 (V):Methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/DK 91/00119**

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on **91-06-27**
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A3- 0095897	83-12-07	AU-D- 1488583	83-12-01
		CA-A- 1196284	85-11-05
		DE-A- 3378523	88-12-29
		JP-A- 58219108	83-12-20
		US-A- 4524075	85-06-18

EP-A2- 0430149	91-06-05	NONE	

